"HISTOPATHOLOGICAL ANALYSIS OF PROSTATIC ADENOCARCINOMA AND THE ROLE OF IMMUNOHISTOCHEMISTRY IN DISTINCTION BETWEEN LOW GRADE AND HIGH GRADE CARCINOMAS"

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CHENNAI

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I, Dr. Nazia Hussain do solemnly declare that this dissertation "**HISTOPATHOLOGICAL ANALYSIS OF PROSTATIC ADENOCARCINOMA AND THE ROLE OF IMMUNOHISTOCHEMISTRY IN DISTINCTION BETWEEN LOW GRADE AND HIGH GRADE CARCINOMAS"** is a bonafide record of work done by me , in the Department of Pathology, Thanjavur Medical College, Thanjavur, under the guidance and supervision of my Professor and Head of Department **Dr. A.L.SANTHI, M.D. (PATHOLOGY) D.G.O,** between June 2015 to June 2017.

This dissertation is submitted to the Dr. M.G.R. Medical University , Chennai, in partial fulfillment of the University's regulations, for the award of M.D. Degree (Branch - III) in Pathology, to be held in May 2018.

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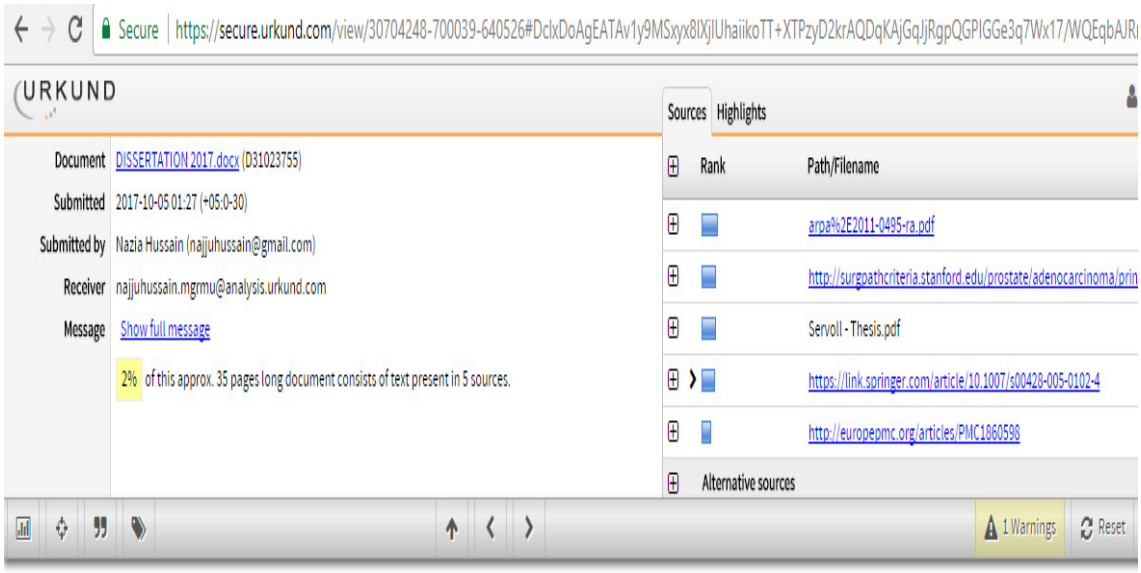

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INTRODUCTION

Prostatic adenocarcinoma accounts for about 95% of malignancies of prostate gland. 7 It is the most common malignant tumor among men. In western countries , it is the second cause of death among all the malignant tumors. Incidence increases from 20% in men in their 50's to 70% in men between ages of 70's and 80's.1 The greatest risk of prostate cancer is among African- American men and it is uncommon among the Asians.1,6,7 The relative distribution of prostate cancer is different in each zone, 68% arises in the peripheral zone, 24% in transitional zone, and 8% in the central zone. 2 Adenocarcinoma of prostate is multifocal in more than 85% of cases.6 Both genetic and environmental factors play a role in the origin of this disease.6 Long term androgen exposure and estrogen receptor β play an important role in the initiation of prostate cancer. Other risk factors include cigarette smoking, fatty diet, obesity, vitamin D deficiency, alcohol consumption vasectomy , PIN, and sexual activity resulting in exposure of prostate to infectious agents , thus increasing the risk of prostate cancer. Green tea, lycopene, soy, vitamin D lower the risk of prostate cancer.7,8 Gleason's grading system is used to grade prostate cancer and it is based on the degree of glandular architectural differentiation and growth pattern of tumor in relation to the stroma.3 It ranges from 2-10 and tumors with grade ranging between 8 -10 are graded as poorly differentiated cancers.1,2,3 Measurement of serum PSA levels helps in the diagnosis and management of prostate cancer though PSA elevations are not always specific for prostate cancer.1,4,6,7 Immunohistochemical markers like cyclin D1 , Ki67 and ER are used to distinguish low grade and high grade prostate cancers. A significant relation is observed between high Gleason's score and increased expression of cyclin- D1 and Ki67.32,33,49 Expression of ER-β is reduced in high grade prostate carcinoma.36,37 Well differentiated prostate adenocarcinomas show positivity for acid mucin with alcian blue

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CONTENTS

INTRODUCTION

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Gleason's grading system is used to grade prostate cancer and it is based on the degree of glandular architectural differentiation and growth pattern of tumo r in relation to the stroma.[3] It ranges from 2-10 and tumors with grade ranging between 8 -10 are graded as poorly differentiated cancers.[1,2,3] Measurement of serum PSA levels helps in the diagnosis and management of prostate cancer though PSA elevations are not always specific for prostate cancer.[1,4,6,7]

Immunohistochemical markers like cyclin D1 , Ki-67 and ER-β are used to distinguish low grade and high grade prostate cancers. A significant relation is observed between high Gleason's score and increased expression of cyclin-D1 and Ki67.[32,33,49] Expression of ER-β is reduced in high grade prostate carcinoma.[36,37]

Well differentiated prostate adenocarcinomas show positivity for acid mucin with alcian blue at p H 2.5 in contrast to benign hyperplasia of prostate which show positivity for neutral mucin.[38,39,40,41]Acid mucin production decreases in high grade prostate carcinomas.

AIM OF THE STUDY

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1. To find out the age distribution of prostate carcinoma reported in the Department of Pathology , Thanjavur Government Medical College from 2015 to 2017.

2. To distinguish between low grade and high grade prostate adenocarcinoma using Gleason's scoring method as well as immunohistochemical markers like Ki-67, cyclin D1, estrogen receptor -β.

3. To demonstrate the production of acid mucin by prostate cancer using histochemical stain like Alcian blue.

4.To correlate the level of prostate specific antigen with the grade of the tumor.

MATERIALS & METHODS

MATERIALS & METHODS

SOURCE OF DATA:

This is a study done in Thanjavur Medical College and Hospital from January 2015 to June 2017. All specimems obtained by transurethral resection of prostate were studied in the Department of Pathology.

METHODS AND COLLECTION OF DATA:

1. Clinical details of patients like age, symptoms, location of the lesion within the prostate gland , serum PSA level were noted and the proforma for collecting case details are given in annexure I.

2. TURP specimens were fixed in 10% neutral buffered formalin , processed in a routine manner and sections were stained with hematoxylin and eosin . The sections were examined under light microscope and using modified Gleason's grading system which is based on the degree of glandular architectural differentiation and growth pattern of tumor in relation to the stroma , the prostate adenocarcinomas were graded into low grade and high grade tumors.

3. Immunohistochemistry was performed on whole tissue section using Cyclin D1, Ki67 and ER . Steps of IHC are given in annexure II.

4.Also special stains like combined alcian blue -PAS were used to demonstrate the tumor morphology and acid mucin production by prostate adenocarcinomas .Combined alcian blue PAS technique is given in annexure III.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Prostate carcinoma is the most common male cancer found in industrialized societies and represents a serious public health problem. [13] It occurs most commonly among elderly men. Peripheral zone of the prostate gland is the most common site to get involved by cancer. The correct diagnosis and grading of prostate cancer is crucial for a patient's prognosis and therapeutic options. Despite significant changes in the clinical and histologic diagnosis of prostate cancer, the Gleason grading system remains one of the most powerful prognostic predictors in prostate cancer.[14] Correct grading of prostate cancer is crucial for patient's prognosis and therapeutic options. The scoring system is named after Donald Gleason ,a Pathologist at the Minneapolis Veterans Affairs Hospital, in the 1960s .[14,15,16,18]Gleason's grading system has remained a cornerstone in the diagnosis and treatment of prostate cancer. [24] It is based on the degree of architectural differentiation and the growth pattern of the tumor in relation to the stroma.[3] Another aspect of this system was , rather than assigning the worst grade as the grade of the carcinoma , the grade was defined as the sum of the two most common grade patterns and reported as Gleason's score.[16]

ORIGINAL GLEASON MICROSCOPIC GRADING SYSTEM FOR PROSTATIC ADENOCARCINOMA: HISTOLOGICAL PATTERNS [3]

Grade 1 – Single, separate, uniform glands in closely packed masses with a definite, usually rounded , edge limiting the area of tumor.

Grade 2 –Single , separate, slightly less uniform glands , loosely packed separated by small amounts of stroma, with less sharp edge.

Grade 3a- Single, separate, much more variable glands ; may be closely packed but irregularly separated , ragged, poorly defined edge.

Grade 3b- Like 3a, but very small glands or tiny cell clusters.

Grade 3c- Sharply and smoothly circumscribed rounded masses of papillary or loose cribriform tumor (“papillary intraductal tumor”).

Grade 4a- Ragged outlined, ragged infiltrating , fused glandular tumor.

Grade 4b- Like 4a, with large pale cells (‘hypernephroid’).

Grade 5a- Sharply circumscribed , rounded masses of almost solid cribriform tumor, usually with central necrosis (comedonecrosis).

Grade 5b- Ragged masses of anaplastic carcinoma with only enough gland formation or vacuoles to identify it as adenocarcinoma.

GLEASON SCORE

The **primary pattern** is the predominant tumor pattern and is graded from 1 to 5 and **secondary pattern** if present is graded similarly and the two numbers are added to obtain **Gleason score or sum.** Tumors with only one pattern are treated as their primary and secondary grades are the same , and hence the number is doubled. Gleason scores range from 2-10, with 2 (1+1) representing the most well differentiated tumors and 10 (5+5) the least differentiated tumors. [1,2,3,4]

In 2005, the International Society of Urological Pathology altered the Gleason system .[16,20,22.] Modified Gleason Score has higher performance than the original one . It is assumed standard in urological pathology.

MODIFIED GLEASON SCORE-2005

Gleason score 1- neoplastic glands are uniform and round in appearance and are closely packed into well circumscribed nodules.

Gleason score 1+1 =2 is extremely rare and most cases were atypical adenomatous hyperplasia. Grade 1 is not reported on needle biopsy. [24]

Gleason score 2- Glands are slightly less uniform i.e. variable in shape , loosely arranged and there is more stroma separating the glands ,such that glands are separated by less than one gland's width. Less circumscribed at periphery , although no infiltration into stroma or between benign glands .Tends to occur more commonly in transition zone and thus should not be diagnosed on needle biopsy. Scores between 2-4 are not usually made on needle biopsy samples.[17]Gleason's pattern 1 and 2 are utilized rarely.[24]

Gleason score 3- Marked variation in size and shape of the glands than seen in grade 2.Typically smaller glands than seen in grade 1 and 2 .Neoplastic glands infiltrate in and among non neoplastic prostate acini. Discrete glandular units , one can draw a circle around each individual gland.

Gleason score 4-Fused microacinar glands

.Ill defined glands with poorly formed glandular lumina.

Cribriform glands.

Glomeruloid glands.

Gleason score 5-

Essentially no glandular differentiation.

Composed of single cells, cords or solid sheets. Comedonecrosis with central necrosis surrounded by papillary, cribriform or solid masses.

However this system continues to have deficiencies and has undergone significant revisions.[14]

Predominant lowest score assigned is Gleason score 3+3=6. [22] Patients who are told that their Gleason score is 6 / 10 may interpret that they have a more aggressive intermediate cancer and experience greater anxiety. Moreover, some classification systems fail to distinguish between Gleason 3+4 =7 and Gleason 4+3=7, with the latter having a worse prognosis.

Epstein JI et al showed large differences in the recurrence rates between cases having Gleason's score 3+4 versus 4+3.[21,22] Thus, new grading system was initially described in 2013 in a study from John Hopkins Hospital and then validated in a multi-institutional study includes five distinct Grade Groups based on the modified Gleason score groups. The modified Gleason grading system was implemented by ISUP in 2005 and subsequent revision was done in 2014. This has an impact on prostate cancer grading and management. .[25] Thus, in 2014,The International Society of Urologic Pathology adopted a simplified grading system composed of 5 prognostic Grade Groups .[18,20,23,24]

GRADE GROUP 1- Gleason score ≤ 6

Only individual discrete well- formed glands.

GRADE GROUP 2- Gleason score 3+4=7

Predominantly well formed glands with lesser component of poorly formed /fused/ cribriform glands .

GRADE GROUP 3- Gleason score 4+3=7

Predominantly poorly formed /fused/ cribriform glands with lesser component of well formed glands.

GRADE GROUP 4- Gleason score 4+4=8; 3+5=8; 5+3=8

-Only poorly formed / fused/ cribriform glands or

- Predominantly well –formed glands and lesser component lacking glands or

-Predominantly lacking glands and lesser component of well formed glands.

GRADE GROUP 5- Gleason score 9-10 (4+5, 5+4, 5+5).

Lack gland formation (or with necrosis) with or without poorly formed/ fused/cribriform glands.

Prostate cancers with a Gleason score ≤ 6 usually have good prognoses. The new grading system and the terminology Grade Groups 1-5 have been accepted by WHO in 2016. [21,23]

GLEASON GRADING

Gleason grading of prostatic adenocarcinoma can be done using 4x objective . In certain instances i.e. cases having back to back glands vs fused glands need higher magnification at 10x objective. [14]

- The Gleason grading system is significantly different from the original version. [14]
- Gleason scores 2-5 is no longer assigned on needle core biopsy, because of poor reproducibility and poor correlation with the radical prostatectomy grade.[14,17.]
- Also Gleason score of 2-5 is misleading for both the clinicians and patients as nearly all cases show higher grade at resection.[14]

- **Thus ,tumors with grades 2 through 6 = well differentiated tumors with excellent prognosis (GRADE GROUP 1)**
- **3+4 = moderately differentiated tumors (GRADE GROUP 2)**
- **4+3= moderately to poorly differentiated tumors (GRADE GROUP 3)**
- **Scores 8 through 10 poorly to undifferentiated tumors with aggressive biologies (GRADE GROUP 4 & 5).**

TERTIARY GRADE ON NEEDLE CORE BIOPSY

In contrast to the original Gleason Grading system, where the primary and secondary patterns were added , it is now recommended that on a needle core biopsy both the most common and the highest grade are added together for the Gleason score , for example if there is 60% Gleason pattern 3, 35% Gleason pattern 4, 5% Gleason pattern 5, the Gleason score would be 3+5=8.[7,14]

Currently established prognostic factors for prostatic carcinoma are TNM stage, surgical margin status, serum PSA and Gleason grade.[19]

Ki-67

Ki-67 protein is also known as **MiB .**It is a cellular marker for proliferation and strictly associated **cell proliferation** Ki-67 antigen antigen can be detected within the **cell nucleus** during **interphase,** whereas in mitosis most of the protein is relocated to the surface of the chromosomes. It is present all active phases of the cell cycle (G1,S,G2,M) but absent in resting cells (quiescent cells-G0).Content of Ki-67 protein increases within the cell during cell progression through S phase of the cell cycle.The fraction of Ki-67 positive tumor cells is known as **Ki-67 labeling index.Ki-67 labeling index** correlates with the clinical course of cancer.

- ***The best studied examples in this context are carcinomas of prostate, breast,nephroblastoma and brain.***

ORIGINAL Ki-67 ANTIBODY

- Monoclonal antibody Ki-67 which was generated by immunizing mice with nuclei of the Hodgkin lymphoma cell line L428.
- The name is derived from the city of origin Kiel, Germany and the number of the original clone in the 96 well plate.

ROLE OF Ki-67 IN PROSTATE ADENOCARCINOMA

Sulik M. et al in 2011 showed that there exists a significant correlation between the Gleason score and the expression of Ki-67 . High expression of Ki-67 was found to be associated with Gleason score of 7 or above . Thus,Ki-67 was found to be a useful tumor marker in prostate carcinoma.[33]

Verma R. et al studied the role of immunohistochemical expression of p53 and Ki-67 as prognostic factor in prostate adenocarcinoma and correlated their expression with Gleason's grade. Prostate fragments were fixed in 10% formalin and embedded in paraffin . H&E stained sections were studied under light microscope and classified into benign and malignant lesions. Carcinoma cases were graded according to Gleason's grading system , and score was noted (well differentiated 2-4, moderately differentiated 5-7, poorly differentiated 8-10). IHC was performed on 4 μm thick sections .Positive and negative controls were run simultaneously . Strong brown nuclear immunoreactivity was considered as positive staining .

Each slide was evaluated at ×40 magnification in order to find areas with maximum positive cells. Then the areas were examined at ×400 magnification and % of positive cells to total cells was calculated. 500 cells were counted. According to the percentage of Ki-67 positive cells the tumors were divided into five groups .

- Cases with Ki-67 index $\leq 2\%$ were considered negative.
- Cases with Ki-67 index $\leq 25\%$ were considered +1.
- Cases with Ki-67 index 26-50% were considered 2+.
- Cases with Ki-67 index 51-75% as 3+ and
- 76-100% were considered 4+.

Ki-67 expression was negative in all well differentiated tumors.61.29% cases with moderately differentiated tumor showed 1+positivity followed by 25.81% cases with 2+ positivity and only 3.22% case showed 3+ positivity. 86.67% poorly differentiated tumors were positive (20% cases with poorly differentiated tumors showed 3+ positivity). [31]

Madani SH et al conducted a study in 2011 to find the frequency of Ki-67 (MIB-1) and P53 expressions among patients with prostate cancer. The results were compared with Gleason's grading. A cross sectional study was conducted using 49 paraffin blocks of prostate cancers. Using Gleason's criteria grades of the tumor were determined.Ki-67 and P53 expressions were determined using IHC staining.The results were analyzed using Spearman's Statistical test.3 / 49 cases (6.1%) were well differentiated , 21 (43%) were moderately differentiated prostate adenocarcinomas and 25 (51%) cases were poorly differentiated tumors.Ki-67 was negative in all well differentiated tumors (28%). Among the moderately differentiated tumours Ki-67 was negative in 8(38%) and among poorly differentiated cases only 3 (12%) cases showed Ki-67 negativity. Thus, a statistically significant relation was observed between increased Ki-67 labeling index and Gleason's grading.

Sasor A et al studied the expression of Ki-67 and P53 in benign and malignant prostatic lesions. Study was conducted in 10 low grade prostatic carcinomas (Gleason's score - 2-4) , 12 intermediate grade prostatic carcinoma cases (Gleason's score - 5-7) and 5 high gradeprostatic carcinoma (Gleason's score-8-10) cases. Although immunopositivity of Ki-67 was found to be increased with the histological grade of prostate cancer the difference in expression between intermediately and poorly differentiated cancers did not statistical significance. Thus , because of the similar level of Ki-67 reactivity in intermediately and poorly differentaited prostate cancer , it was stated in this study that both grades of prostate adenocarcinoma had similar biology.[34]

CYCLIN D1

Cyclin D1 is a protein that is encoded by the CCND1 gene in the humans.Human CCND1 gene is located on 11q13 . Cyclin D1 is expressed in all adult human tissues with the exception of cells derived from bone marrow stem cell lines. Cyclin D1 forms a complex with CDK4 , whose activity is required for G1/S transition in the cell cycle. CyclinD1-CDK4 inhibits pRb through phosphorylation allowing E2F transcription factor to transcribe genes required for entry into the S phase. Inactive pRb allows cell cycle progression through G1/S transition and allows for DNA synthesis. Cyclin D1 overexpression correlates with early cancer onset and tumor progression.

ROLE OF CYCLIN D1 IN PROSTATE CANCER

Cyclin D1 is a short lived nuclear protein and it is degraded by the ATP ubiquitin-dependent proteolysis pathway. This protein is involved in cell cycle transition from G1 to S phase in both normal and neoplastic cells. Overexpression of cyclin D1 causes uncontrolled cell proliferation, and transformation to a neoplastic phenotype , thereby acting as an oncogene.

Some studies have shown that expression of cyclin D1 cancer is rare,whereas others report that prostate tumors with high cyclin D1 expression are associated with more aggressive disease.

Pereira et al studied the expression of cyclin D1 in prostate adenocarcinoma in 2014. 450 patients with prostate carcinoma were identified , of which a total of 156 were analysed. From these 43 cases with high grade Gleason score and 42 cases with a low grade Gleason score were randomly selected. Tumors were graded as low grade (Gleason score $\leq$ 6) and high grade (Gleason score $\geq$ 7). IHC was performed on 4μm tissue sections using a polymer based method and antigen retrieval. Cyclin D1 monoclonal antibodies were used. The sections were immersed in EDTA tris solution at a pH 9.0 for 30 mins at 95 degree celsius. After washing the endogenous peroxidase activity was blocked and also non specific antibody sites were blocked .The sections were then incubated with antibodies to cyclin D1 for 2 hours. DAB chromogen was used and hematoxylin was used as a nuclear counterstain.

Tumor samples with atleast 2000 cells were counted at 200× magnification . Cells showing nuclear positivity were counted, irrespective of the intensity.A significant association was found between cyclin D1 expression and Gleason score . High grade tumors displayed high cyclin D1 expression compared to low -grade tumors . [48]

ESTROGEN RECEPTOR

Estrogen receptors are a group of proteins found both inside and on cells. These receptors are activated by the hormone 17β - estradiol. There are 2 classes of estrogen receptors:

Nuclear estrogen receptors (ERα and ERβ)

Membrane estrogen receptors (GPER, ER-X and Gq-mER) .After being activated by estrogen , the ER gets translocated into the nucleus and binds to DNA , thus regulating the activity of different genes.ER α ,ER β receptors are encoded by ESR1 and ESR2 genes on 6q25.1 and 14q22.24 respectively. Estrogen has an important role in prostate carcinogenesis. The prostate expresses both ER alpha and ER beta .

ER alpha mediates harmful effects of estrogen in the prostate , whereas ER beta is tumour suppressive.[38] Loss of ER beta leads to uncontrolled growth of prostate epithelial cells.[37]

ROLE OF ERβ IN PROSTATE ADENOCARCINOMA

Androgen receptor signals regulate the growth and function of the prostate gland .ERβ is involved in the differentiation of prostatic epithelial cells and it also has antiproliferative actions on prostate cancer cells. ER-b has promising anticancer properties and helps in preventing prostate cancer.

Nobelists Huggins and Hodges provided clinical evidence that androgens promote tumor growth and estrogens inhibit it. [39]

Christoforou P. et al showed that estrogen has the ability to decrease hypothalamic pituitary stimulation of leutinizing hormone and follicle stimulating hormone production and consequently reduce androgen synthesis and demonstrated the therapeutic role of estrogen in prostate cancer. [39] ERβ protects epithelial integrity and blocks EMT by upregulating transcription of E-cadherin . [39]

ERβ signaling enhances the expression of FOXO3α and also upregulates apoptotic genes like PUMA , a proapoptotic protein and p21, a regulator of cell cycle progression ,thus has antiproliferative effects on the prostate.[39] Additionally ERβ maintains differentiation by causing degradation of hypoxia-inducible factor 1α (HIF-1α). ERβ enhances the transcription of prolyl hydroxylase domain-30 containing protein 2 (PHD2) and hydroxylates HIF-1α. Hydroxylation of HIF-1α marks HIF for destruction by the von Hippel Lindau tumor suppressor (VHL). [40] The antiproliferative role of ERβ was confirmed with immunohistochemical findings in prostate cancer tissue samples. ERβ expression was lost in high grade tumors when compared to low grade and intermediate grade tumors. [35 ,37] Thus, loss of ERβ was found to be a prognostic factor of prostate cancer.

ERβ and oxidative stress:

Oxidative stress is associated with aggressive phenotypes of prostate cancer . Whereas antioxidants have a role in chemoprevention of prostate cancer.

Cell lines with high amounts of ERβ have high expression of anti-oxidants enzymes , resulting in lower oxidative stress. Origin of oxidative stress in prostate cancer include mitochondrial H2O2 production through cytochrome c oxidation . Oxidative stress inactivates ERβ by inhibiting dimerization of the the receptor and thus destabilizing its DNA binding capacity. Thus, ERβ loses its ability to regulate the genes.[39]

ERβ isoforms during prostate cancer progression:

Different types of ERβ exist. ERβ 2 promotes cancer cell migration by inducing the expression of factors involved in bone metastasis. ERβ2 is not only associated with EMT but also has the ability to suppress ERβ 1 expression.

Nagasaki et al indicated a correlation between immunoreactivity of gastrin releasing peptide receptor , Gleason score and ERβ 2 and hypothesized that ERβ 2 contributes to prostate carcinogenesis through GRPR expression in PCa cells. Thus, ERβ 2 and ERβ5 promote cell migration and invasion and act as cancer enhancing molecules.[39]

ERα and EMT:

High grade prostate cancer cells lose their epithelial characteristics and exhibit mesenchymal features such as loss of E-cadherin , increased expression of VEGF,vimentin, HIF-1α .All are events typical of EMT phenomenon. 3β -androstanediol is a natural ligand of ERβ and promotes binding of dimerized ERβ to DNA promoter of E-cadherin, thus stimulating its transcription. Exposure to hypoxia and TGF β can induce EMT and reduce ERβ expression in both AR dependent and independent cells. Thus, hypoxic conditions and TGFβ 1 signaling diminishes ERβ levels, favoring prostate cancer progression. [39]

ER β agonists:

A SERM , named ICI 182,780 exerts dose dependent growth inhibition action on DU145 cells. The action is mediated by ERβ through binding to NF-kβ and enhancement of transcription factor FOXO1.Also Raloxifene, another SERM also induces apoptosis via activation of ERβ lowering Bcl-2 expression and increasing caspase 3.[39]

Imamov O et al stated that ER-β suppresses proliferation and promotes differentiation of prostate epithelial cells.

Bosland MC stated that estrogens , particularly diethylstilbestrol , are effective against androgen dependent prostate cancer , but paradoxically estrogens might also be involved in the causation of this malignancy. Therefore, antiestrogens have been suggested as both a chemopreventive and chemotherapeutic treatment, thereby inhibiting the development and progression of prostate cancer. DES suppresses secretion of leuteinizing hormone from the anterior pituitary gland and thus inhibits testosterone production from the testis. Thus, DES acts as an antiandrogen.ER -α and ER-β are expressed in the normal prostate . ER-α is found in the stromal cells of the human prostate and ER-β is present in the basal cells. ER-β expression is decreased during malignancy progression due to methylation of CpG dinucleotides in the promoter of the gene. Thus , this suggests that ER-β has a function in tumor suppression. Tamoxifen, a SERM inhibits the proliferation of PC-3 and DU-145 prostate cells and induces apoptosis in LNCaP.Raloxifene is also found to have anti-prostatic tumor effect.[42]

Asgari M . et al investigated the expression of ER-b in human prostate cancer tissues. 52 paraffin embedded blocks of TRUS guided prostate needle biopsies diagnosed as prostate carcinoma and related H&E slides and pathology reports were retrieved from archive of pathology of "Hasheminejad Kidney Center". All the 52 specimens were reviewed by the Pathologists and they found the tissues to be sufficient for IHC analysis. Tissue sections were cut 4 to 6μm thick and stained by routine H&E. These slides were reviewed by Pathologists to confirm the diagnosis and histologic grade was assigned based on Gleason grading system criteria. Tumors with Gleason score ≤ 4 were considered as low grade, those with score of 5,6 and 7 were considered as intermediate grade and those with Gleason score ≥ 8 were considered as high grade tumors. The sections were deparaffinised and rehydrated . This was followed by epitope retrieval .Sections were cooled at room temperature . Then they were rinsed in water and placed in Tris-buffered saline. Sections were then incubated with peroxidase block for 5 minutes to neutralize the endogenous peroxidases .

Then the sections were incubated at room temperature with mouse anti-human ER-b ;clone PPG5/10 for 30 minutes. Rate of ER b expression was defined as % of positive nuclei per 200 cells that were counted. 100% of low and intermediate grade cancers and 83% of high grade cancers were positive for ERβ and 17% of high grade cancers were negative for ERβ expression.[35] Al-Magrabi JA et al conducted a study in 2010 to analyse the expression of ERα and ERβ receptors in prostate cancer and hyperplasia . Among the 65 cases of PCa , ERα was expressed in 3 cases in epithelial cells and 4 cases in stromal cells. ERα was not expressed in any of the HGPIN foci and also was not expressed in both in the luminal or basal cells in all the BPH cases. However , it was expressed in stromal cells in 4 cases (11.4%) of BPH. ERβ was expressed in 61 cases (93.8%) in epithelial cells and 35 cases (53.8%) in stromal cells respectively. Out of 7 HGPIN cases only 2 cases showed ERβ expression. Also ERβ was expressed in 33 cases of epithelial and stromal cells of BPH. Thus, it was concluded in this study that majority of PCa and BPH exhibited nuclear immunoreactivity for ER-β in both tumor and stromal cells and were usually negative for ER-α. There was also partial loss of ERβ in HGPIN. Thus , it proved that ER-β was found to have a role in prostatic hyperplasia and malignancy.

Horvath LG et al also stated that there is frequent loss of ER-b expression in prostate cancer. Five normal prostates from organ donors and 159 radical prostatectomy specimens from patients with prostate cancer were assessed for ER beta expression using immunohistochemistry. All 5 normal prostates showed nuclear staining for ER beta in > 95% of the epithelial cells and 35% of the stromal 35 cells. The number of ER beta positive cases declined to 24.2% in hyperplasia adjacent to carcinoma and 11.3 % in prostate cancers .Thus, with progression from normal prostate epithelium to prostate cancers loss of ER-b expression was demonstrated.[36]

ROLE OF HISTOCHEMISTRY IN PROSTATE ADENOCARCINOMA.

Prostate adenocarcinomas produce acid mucin in contrast to benign cases and this can be demonstrated by alcian blue at pH 2.5. PAS positivity can be demonstrated in majority of the benign and malignant lesions. Different types of mucin are present in tissues or are secreted by the glands. Alcian blue at pH 2.5 is used to study acid mucin , PAS stain is used to demonstrate neutral mucin and combined Alcian blue -PAS to study the mucin character.[45]

Agarwal D.N. et al evaluated the usefulness of mucin stains to differentiate between benign and malignant lesions of prostate. 30 cases of prostate carcinoma were chosen. Sections were stained with PAS- Alcian blue (2.5 pH) and combined Alcian blue - PAS to study the mucin character. All the cases of prostate carcinoma showed positivity for acid mucin in addition to neutral mucin. All low grade prostate carcinomas showed positivity for acid mucin but none of the high grade carcinomas showed acid mucin positivity.[45]

Pinder et al and McMohan RF demonstrated acid mucin positivity in 38% and 50% of prostate carcinoma cases respectively.[47]

Arora HL demonstrated acid mucin positivity in 60% of prostate cancer cases.[46]

Khanna A et al conducted a cohort study in 176 patients . Alcian bluepositivity for acid mucin was observed in 66.67% of prostate adenocarcinoma cases. The intensity of positive reaction of the Alcian blue varied from deep blue near the mucinous areas and light blue in areas without mucinous differentiation.[43]

Mathur SK also showed the significance of mucin stain in prostate lesions using 200 biopsies. Acid mucin was found in prostate carcinoma cases (68%). The positivity was more in well differentiated tumors and was found to be decreased in high grade malignancies.

CORRELATION OF SERUM PSA LEVELS WITH TUMOR GRADE

PSA is a 34 -kDa single chain glycoprotein . It consists of 237 amino acids and is produced exclusively by prostatic epithelial cells.[7] PSA is a serine protease and belongs to a member of the kallikrein gene family.[6] In the serum PSA is mainly present as a complex with α1 - antichymotrypsin.[6] Only a small amount of PSA in semen is complexed . The free non-complexed form of PSA constitutes only minor fraction of serum PSA and does not form complex with antiprotease. [6] Production of PSA is under the control of circulating androgens acting through androgen receptors. PSA is used for early detection of prostate cancer. The goal of screening is to identify the disease and give treatment in order to prevent cancer related death and suffering . [28] Serum PSA is also elevated by conditions other than cancer, including prostatitis, PIN, infarction of nodular hyperplasia, acute urinary retention and renal failure.[6,26]

Also manipulation of prostate gland like prostate massage , prostate biopsy can cause elevation of PSA levels to variable degrees.[26] DRE performed in outpatient setting can also lead to increase in S. PSA level. PSA is sensitive and accurate in detection of prostate volume, residual cancer, recurrence and cancer progression following treatment. There are multiple derivatives of PSA as adjuncts for total PSA like free PSA, conjugated PSA, age specific reference ranges, PSA density, PSA velocity, proPSA, BPSA. Serum PSA levels vary with age, race and prostate volume in the absence of prostate cancer. Disruption of cellular architecture within the prostate gland results in elevation of serum PSA levels. Loss of barrier that is provided by the basal layer and basement membranes within the normal prostate gland is a site for the egress of PSA into the circulation. [6] PSA elevations indicates the presence of prostate disease , but not all men with prostate disease have elevated PSA levels. Also PSA elevations are not always specific for prostate cancer. Therapeutic measures like orchiectomy, leutinizing hormone releasing hormone analogs , 5α reductase inhibition, radiotherapy and surgery for BPH and prostate cancer can all result in reduction in serum PSA level.

Prior to treatment with Finasteride (5mg) , baseline serum PSA level should be measured followed by serial measurements of PSA .

This drug reduces the serum PSA levels by 50%. The patient should be suspected of having occult prostate cancer if the serum PSA level does not decrease by 50% or if there is a rise in the serum PSA level when the patient is taking finasteride.[6]

S.PSA LEVELS:

PROBABILITY OF PROSTATE CANCER BY TOTAL S .PSA[6]

Total PSA < 4 ng/ml - < 2%
- < 2.5 ng/ml - < 2%
- 2.5 ng/ml -4.0 ng/ml ~ 18%

Total PSA 4-10 ng/ ml ~ 25 %

Total PSA > 10 ng/ml ~ 67%

Range between 2.5 -10 ng/ml is considered as "gray zone".

PSA DENSITY

It is defined as **S.PSA/ Prostate volume .** In men with PSA levels between 4.0 -10.0 ng/ml and normal DRE , **PSAD > 0.15** is associated with the presence of **prostate cancer.**[6]

PSA VELOCITY

The rate of change in the serum PSA value with time best distinguishes men with and without prostate cancer**.**

Rate of change in the serum PSA > 0.75 ng/mL per year is a specific marker for the presence of prostate cancer.

Men with prostate cancer have significantly more rapid rise in PSA levels than men without cancer.[6]At least three PSA measurements must be performed over a period of 1.5 -2 years.[1]

AGE -SPECIFIC SERUM PSA LEVELS [1]

For men between

40-49 years --> 2.5 ng/mL

50-59 years --> 3.5 ng/mL

60- 69 years --> 4.5 ng/mL

70-79 years --> 6.5 ng/mL

Disadvantages:

1. Risk missing significant cancer in older men.
2. Over detection in younger men.

FREE AND THE PERCENT FREE PSA

Although majority of serum PSA is complexed to ACT , 5%-35% of PSA exists as fPSA. The prostate cancer cells do not produce more PSA than benign prostate epithelium . But PSA produced from malignant cells escape proteolytic process . Thus , men with prostate cancer have greater fraction of PSA that is complexed to ACT when compared to men without prostate cancer. Measurement of the percentage of the ratio of free to total PSA (%fPSA) provides an additional degree of specificity for prostate cancer detection.

%fPSA is greater in men without prostate cancer who have prostate This difference is because of differential expression of PSA isoforms by transition zone (zone of BPH) compared with peripheral zone (zone of prostate cancer). %fPSA is approved by FDA for use to aid PSA testing in men with benign DRE and minimal PSA elevations , within the diagnostic gray zone of 4-10 ng/mL. %fPSA helps to counsel men with PSA elevation between 4-10 ng/mL regarding their risk of cancer and need for further evaluation to rule out the disease.[6] Free PSA levels are usually lower in prostate cancer than in BPH cases.[26] Thus, higher total PSA and lower % fPSA is associated with higher risks of cancer. The probability of finding prostate cancer on needle biopsy by age in years reduces when free: total PSA >0.25.[26]

COMPLEXED PSA

Complexed forms of PSA are bound to ACT (alpha -1-anti chymotrypsin), and to a lesser extent α1 Protease Inhibitor (API) . The sum of these and other unknown PSA complexes is given the term complexed PSA (cPSA).[6]

Complexed PSA has been shown to improve the specificity in the detection of prostate cancer over that of tPSA testing in men with tPSA values greater than the cut off value of 4.0 ng/mL.

Serum isoforms of free PSA.

Precursor form of PSA contains a 7- aminoacid proleader peptide in addition to the 7 constituent amino acids of mature PSA and is termed as pPSA. Pro PSA is released into the lumen , where 7-aminoacid leader chain is cleaved by hK2 to yield active PSA. This active PSA diffuses from the lumen into the serum and gets bound to proteases such as alpha1 - antichymotrypsin. In the lumen the active PSA undergoes proteolysis resulting in the formation of inactive PSA (iPSA). Thus, both active PSA and iPSA may enter the circulation and iPSA circulates in free or unbound state in contrast to active PSA that circulates in bound form. The PSA produced by malignant cells does not undergo proteolysis . In prostate cancer, loss of the tissue architecture permits a relative increase in bound PSA and proPSA in serum. In addition to undergoing proteolysis active PSA may also undergo internal degradation to form benign PSA (BPSA). BPSA represents a cleaved form of PSA that is identified in tissue from the nodular BPH transition zone tissue.

Partial cleavage of 7-amino -acid-leader sequence yields inactive form of pro PSA ([-2]pPSA or [-4]pPSA).[6]

CORRELATION OF SERUM PSA LEVELS WITH GLEASON SCORE

Shih W.J. et evaluated the relationship between Gleason scores of prostate carcinoma and concurrent serum PSA values. 65 men with prostate carcinoma were studied and Gleason scores were obtained by summing up the primary and secondary patterns. These patients were divided into 2 groups .42 patients received high (6-10) and 23 received low (2-5) Gleason scores. Serum PSA was measured by radioimmunoassay 1-7 days before surgical procedure or biopsy. Mean serum PSA for patients in the high Gleason score group was 134.39 ng/mL and the mean serum PSA for patients in the low Gleason score group was 23.62 ng/mL. Thus, there was a significant correlation between Gleason's score and serum PSA levels.[29]

Salgaonkar et al showed that all patients with prostate cancer who had bone metastasis on scintigraphy had PSA value > 20 ng/mL and only one patient with bone metastasis had PSA value <50ng/mL. In this study no significant correlation was found between PSA level and tumor grading by Gleason score.[27]

Issac AS et al conducted a study in 2014. 16 prostate adenocarcinoma cases were selected .4/16 cases with well differentiated adenocarcinomas had low serum PSA levels. 3/4 well differentiated adenocarcinoma cases had normal serum PSA levels and the remaining 1/4 cases of well differentiated tumors had a borderline serum PSA level of 8.8 ng/mL12 cases had high Gleason score ranging between 6-9. All the 12 cases with high Gleason's score had serum PSA level > 100 ng/mL with a mean of 208 ng/mL.[30]

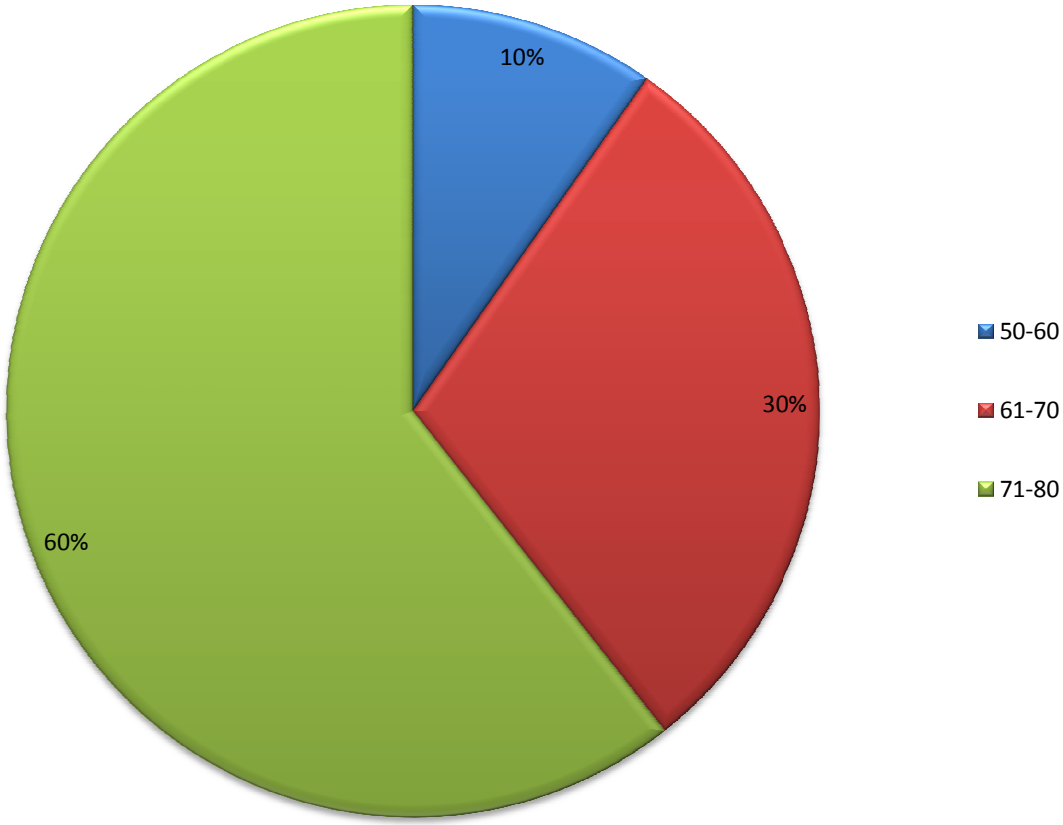
OBSERVATION AND RESULTS

OBSERVATION AND RESULTS

1. AGE DISTRIBUTION OF PROSTATE ADENOCARCINOMAS:

During the study period from January 2015 to June 2017, a total of 50 cases of prostate adenocarcinomas were received . Of these 50 cases , 5 cases (10%) were between the age group of 50-60 , 15 cases (30%) were between 61 -70 and the remaining cases 30 (60%) were between the age group of 71-80 . Age distribution of prostate cancer cases is depicted in figure 1.

Figure 1. AGE DISTRIBUTION



2. SITEWISE DISTRIBUTION OF PROSTATE ADENOCARCINOMAS

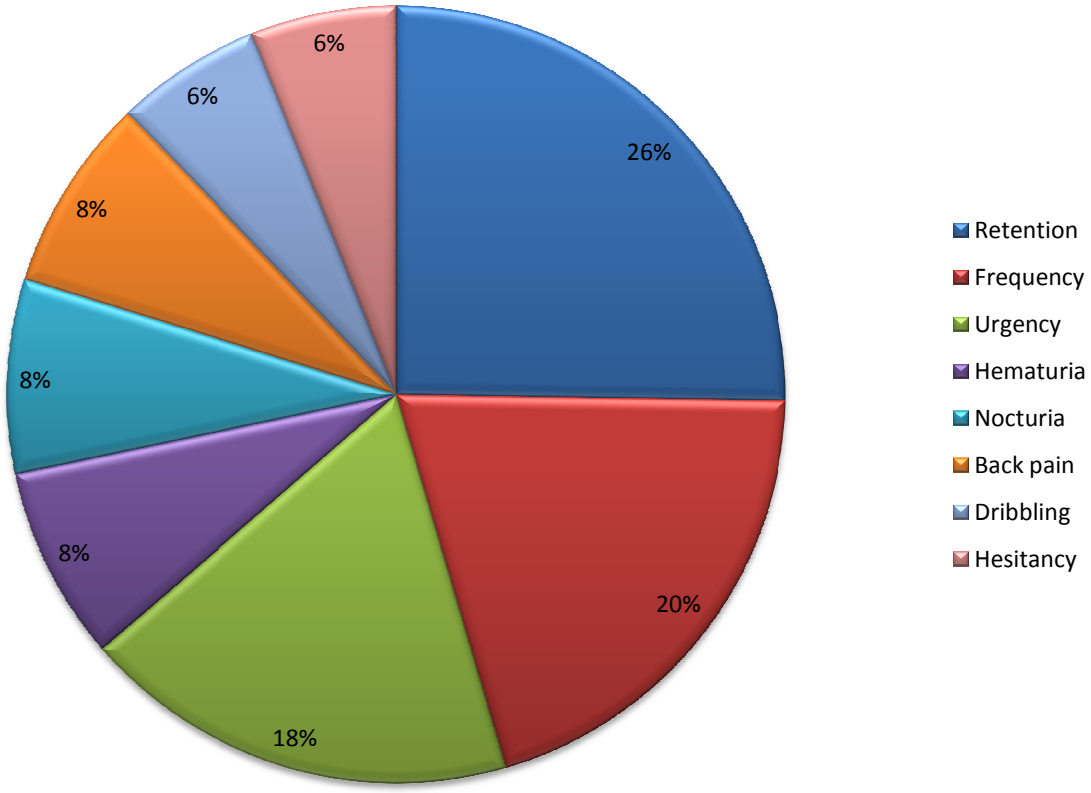
Of the 50 cases studied , all the 50 cases (i.e. 100%) of the prostate adenocarcinoma involved the peripheral zone (posterior lobe) of the prostate. Site wise distribution of prostate cancer cases is depicted in figure 2 below.

Figure 2. SITEWISE DISTRIBUTION OF PROSTATE ADENOCARCINOMA

3. SYMPTOMWISE DISTRIBUTION OF PROSTATE ADENOCARCINOMAS

Symptom wise distribution of prostate adenocarcinomas is depicted in the figure below (fig.3) .Maximum number of cases in this study had retention of urine (13/50 cases -26%) followed by increased frequency of urination (10/50 cases-20%), urinary urgency (9/50 cases-18%), hematuria(4/50 cases-8%),back pain (in 4/50 cases -8%), nocturia (4/50 cases- 8%), hesitancy of urine (in 3/50 cases-6%) and dribbling of urine (3/50 cases-6%).

Figure 3. SYMPTOMWISE DISTRIBUTION OF PROSTATE ADENOCARCINOMA



4. DISTRIBUTION OF PROSTATE ADENOCARCINOMA ACCORDING TO GLEASON'S SCORE

Distribution of prostate adenocarcinoma according the Gleasons score is depicted in the figure below (fig .4). 13 cases (26%) had Gleasons score of (3+2) 5, 12 cases , 11 cases (22%) had a score of (3+3) 6 , 10 patients had a Gleason score of 7 (20%), 6 (12% of the cases) had a score of 9, 5 (10%) cases had a Gleason score of (2+2) 4 , 3 (6%) cases had a score of (2+1) 3 and 2 cases (4%) had a score of (1+1) 2.

Out of the 6 (12%) cases that had the highest Gleason's score of 9 , 4 (8% cases) had a score of (4+5 = 9) and the other 2 (4% cases) had a score of (5+4=9).And of the 10 cases that had a score of 7, 8 (16%) had a score of 3+4 and 2 (4%) of the cases had a Gleason score of 4+3.

Cases with ;

Gleason score 2 through 6 were grade group 1 tumors which were well differentiated adenocarcinomas.

Gleason score 3+4=7 were clumped into grade group 2 tumors which were moderately differentiated adenocarcinomas.

Gleason score 4+3=7 were grade group 3 tumors that were considered moderately to poorly differentiated adenocarcinomas.

Gleason scores 8 through 10 were poorly to undifferentiated tumors with aggressive behavior and included grade group 4 & 5.

Thus 34/50 (68%) cases had well differentiated adenocarcinoma, 8/50 (16%) cases had moderately differentiated , 2/50 (4%) had moderately to poorly differentiated adenocarcinoma and the remaining 6/50 (12%) cases had poorly to undifferentiated prostate adenocarcinoma . This is shown in figure.5 below.

Figure 4. DISTRIBUTION OF PROSTATE ADENOCARCINOMA ACCORDING TO GLEASON'S SCORING

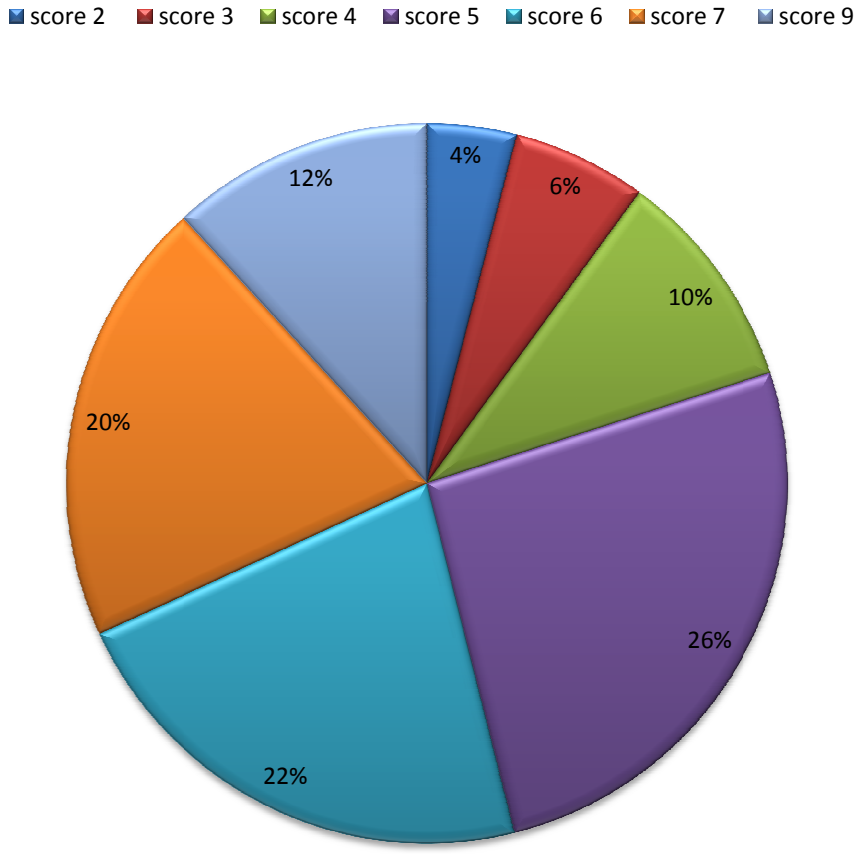
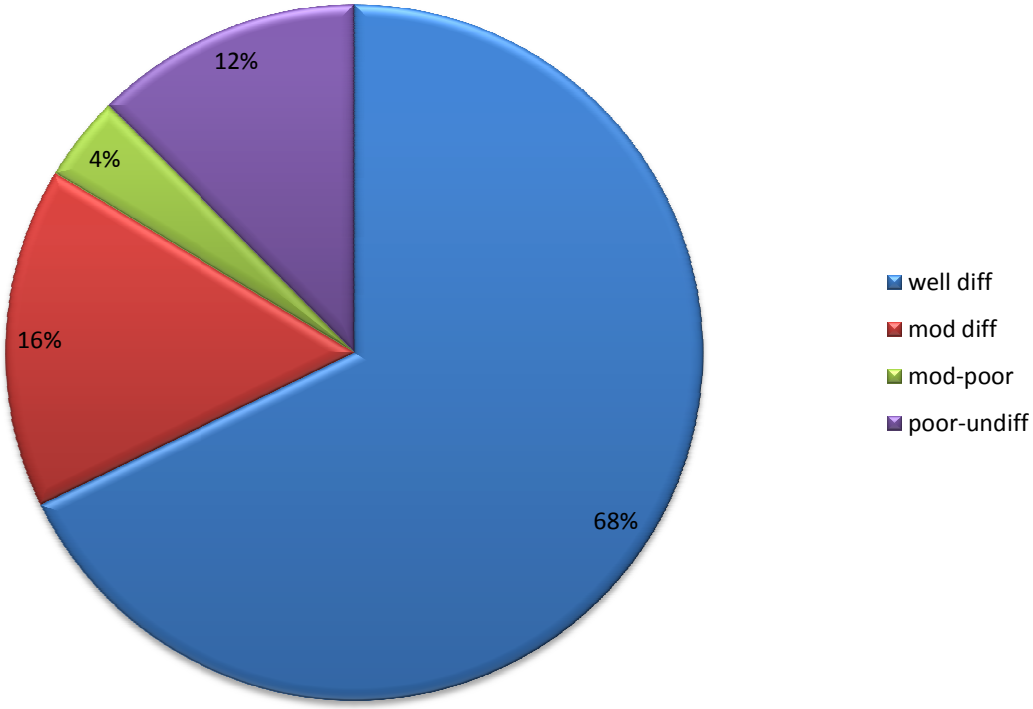


FIG.5 DISTRIBUTION OF DIFFERENT GRADES OF PROSTATE ADENOCARCINOMA ACCORDING TO MODIFIED GLEASON'S SCORING SYSTEM

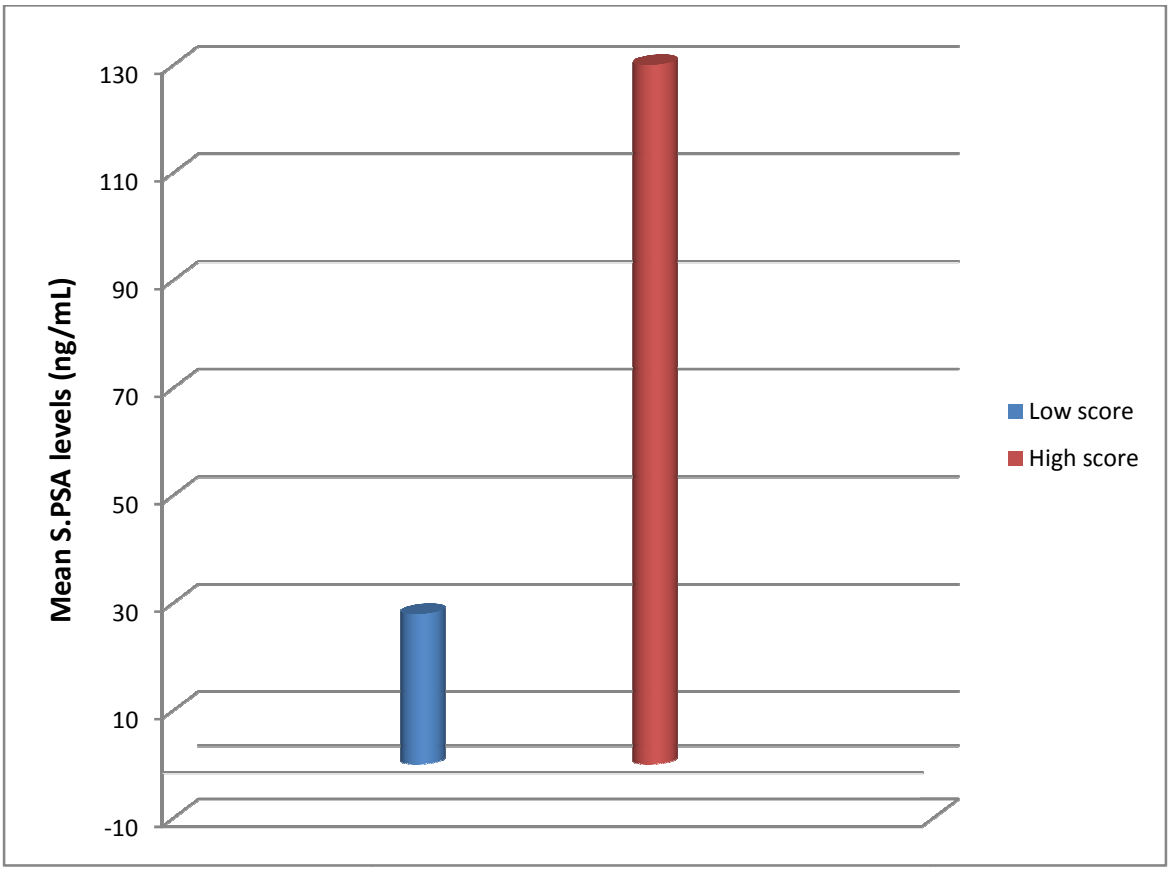


5. CORRELATION OF S.PSA WITH GLEASON SCORE

Correlation of serum PSA levels with different grades of prostate adenocarcinoma has been depicted in figure .6 .

Of the 50 cases , patients with low Gleason score (2-5) had serum PSA level ranging from 15-50 ng/mL, with a mean value of 27.9 ng/mL Cases with high Gleason score (6-10) had a serum PSA levels ranging from 80 - 280 ng/mL , with a mean serum PSA level of 139.25 ng/mL.

FIG.6 :CORRELATION OF S.PSA LEVELS WITH GLEASON SCORE

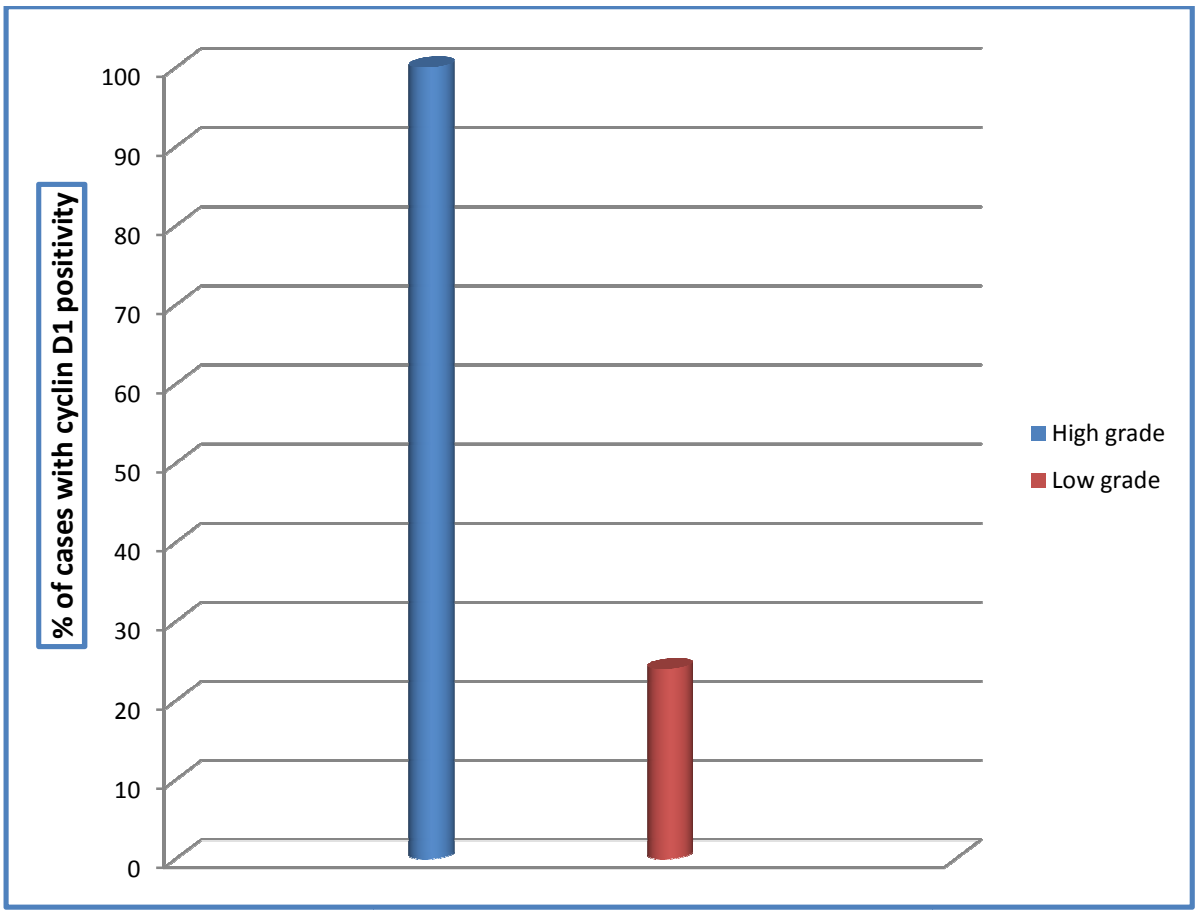


Thus, the mean expression of serum PSA level increases with increase in the grade of the tumor.

CYCLIN D1 EXPRESSION IN HIGH GRADE AND LOW GRADE PROSTATE ADENOCARCINOMAS

A cut off value 5% was used to determine cyclin D1 positivity i.e. cases in which > 5% of the tumor cells were stained with cyclin D1 are considered to be positive for cyclin D1. 16/50 cases had high grade prostate adenocarcinomas (Gleason score ≥7) .All the 16 (100%) high grade prostate adenocarcinoma cases showed cyclin D1 positivity i.e. >5% of tumor cells were stained with cyclin D1. The remaining 34 cases of low grade prostate adenocarcinomas had a score of ≤ 6 . Of these 34 low grade tumours only 8 (23.5%) cases showed cyclin D1 positivity. The remaining 26 (76%) of low grade adenocarcinomas showed no immunopositivity with cyclin D1. The mean expression of cyclin D1 in high grade tumors was 32.5% and 15% in low grade tumors. Figure 7 shows the difference in the number of cases showing cyclin D1 expression between low and high grade tumors.

FIGURE 7- PROPORTION OF HIGH AND LOW GRADE TUMORS SHOWING CYCLIN D1 EXPRESSION



Thus, large number of high grade tumors show Cyclin-D1 expression.

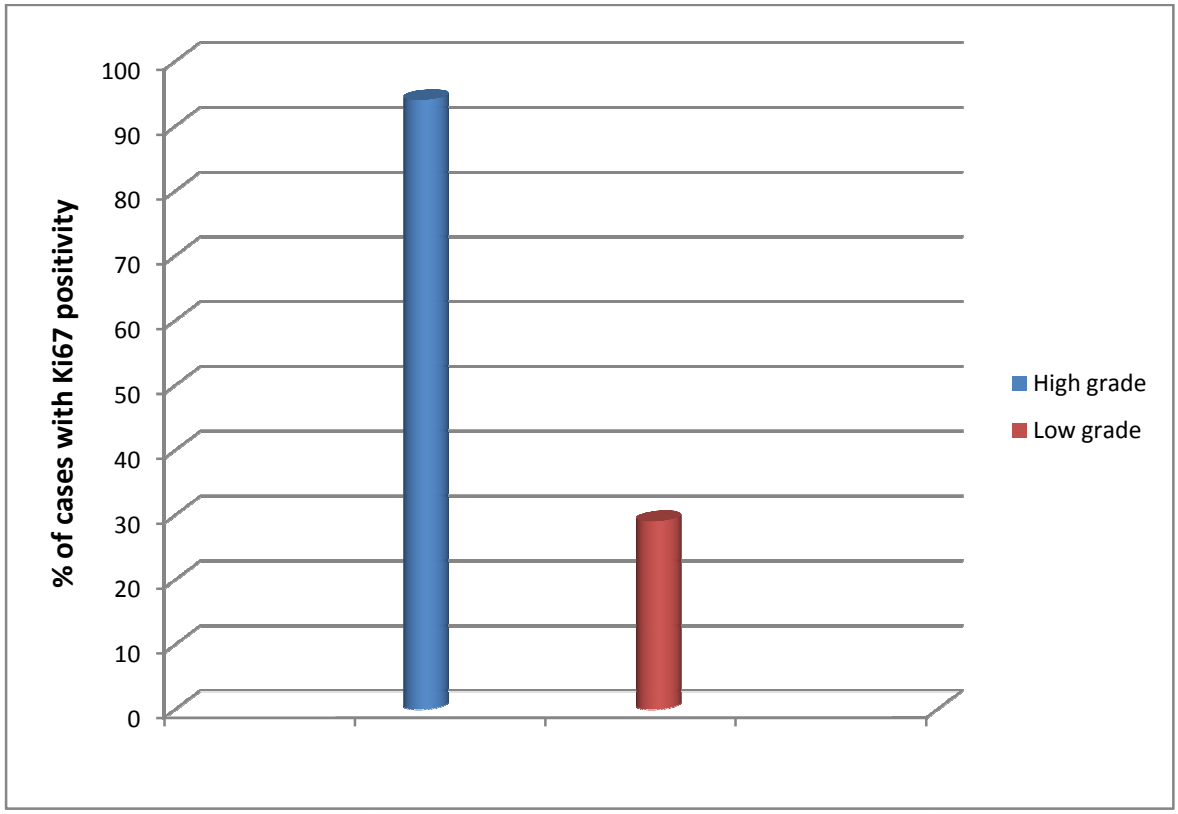
Ki-67 EXPRESSION IN HIGH AND LOW GRADE PROSTATE ADENOCARCINOMA

Ki-67 is a nuclear stain . IHC was done using Ki-67 monoclonal antibody in all 50 cases . Cases in which $\leq 2\%$ of tumor cells were stained with Ki67 were considered negative. Cases with Ki-67 index between 2% - 25% were considered 1+ , 26%-50% as 2+ , 51-75% as 3+ and 76-100% as 4+.

In the present study , 16/50 cases were high grade tumors (Gleason score ≥ 7) and 34/ 50 grade tumors had a Gleason's score ≤ 6 .8 cases with Gleason's score 7 had Ki-67 index 2-25% and 1 case with the same score had Ki-67 index ranging between 26- 50%. 4 cases with Gleason score 9 had an index of 26-50% and 2 cases had a higher Ki67 index between 51-75%.

15/16 high grade tumors i.e . 94 % showed Ki-67 positivity . Only 10/34 (29%) low grade prostate adenocarcinoma cases showed Ki-67 positivity and the remaining 24 cases (70.58%) with low grade carcinoma did not show Ki-67 expression. % of low and high grade tumors showing Ki-67 expression is depicted in figure 8 below.

FIGURE 8- Ki-67 EXPRESSION IN HIGH AND LOW GRADE PROSTATE ADENOCARCINOMAS

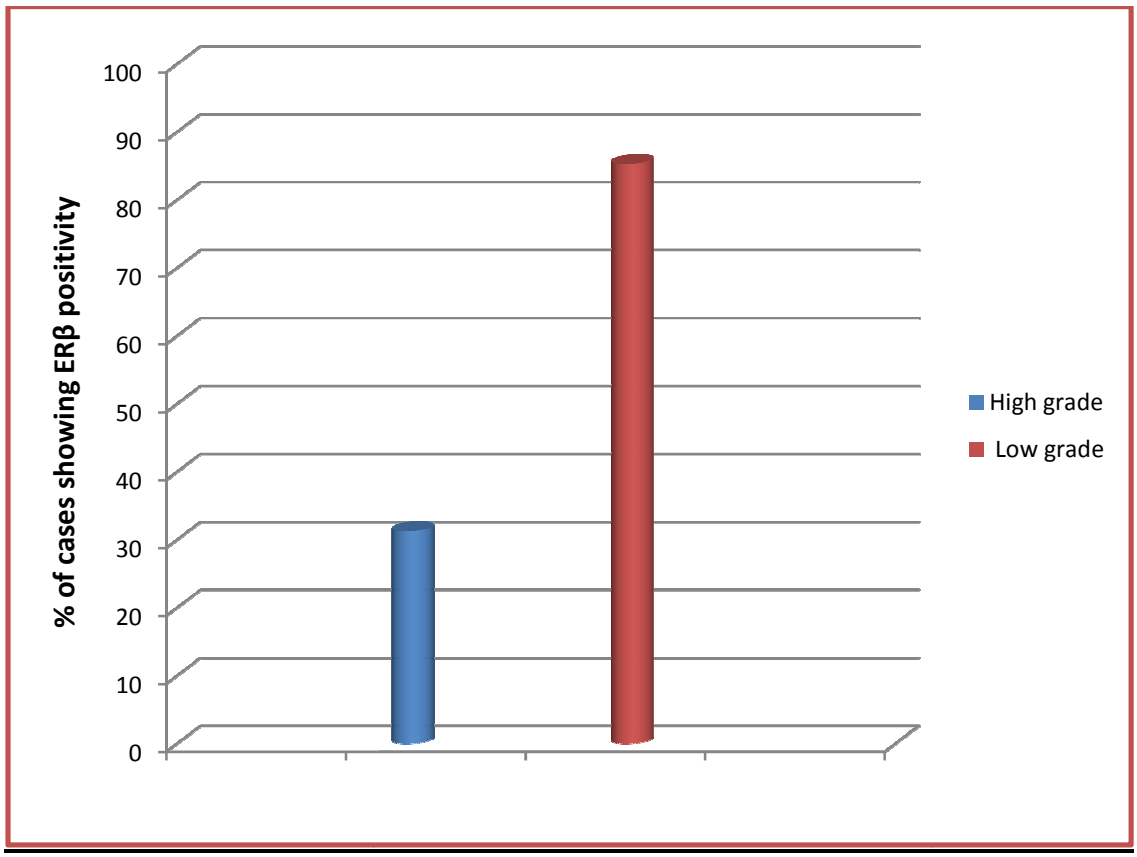


Thus, large number of cases with high grade tumors show Ki-67 expression.

ER β EXPRESSION IN PROSTATE ADENOCARCINOMA

Among the 16 high grade tumours only 5 (31.25%) of the cases showed ER β positivity and the remaining 11 cases were negative for ER-b .Among the 34 low grade tumors 29 (85.29%) cases showed ERβ expression . Mean expression of ER-b in low grade tumors was found to be 30.58% and in high grade tumors was 7%. Figure 9 shows the percentage of cases showing ER-b expression.

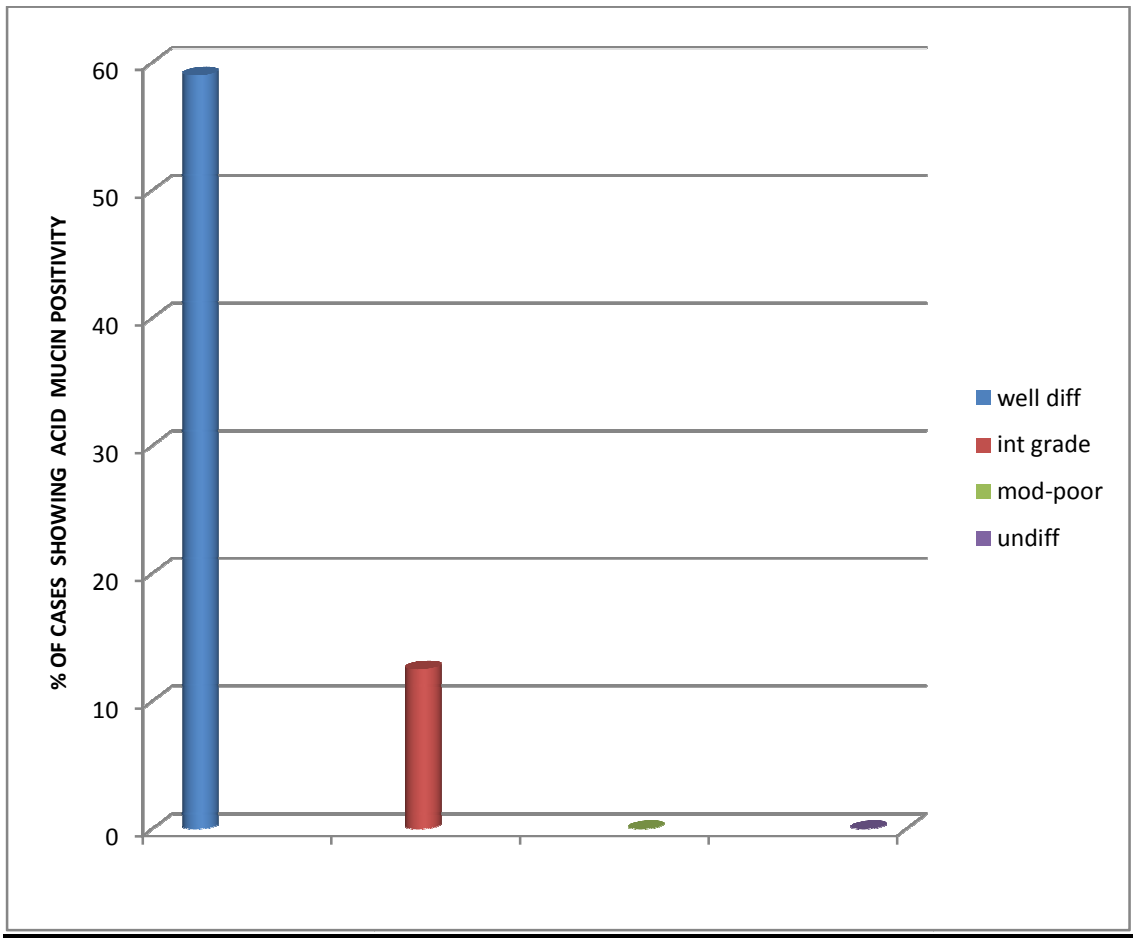
FIG 9. PROPORTION OF CASES SHOWING ER-b EXPRESSION



ACID MUCIN POSITIVITY IN PROSTATE ADENOCARCINOMA

Alcian blue stain was used to demonstrate the production of acid mucin by prostate adenocarcinomas. 34/ 50 are low grade cancers. Out of these 34 cases , 20 (59%) low grade tumors showed alcian blue positivity. Among the 8/50 intermediate grade tumors , with a Gleason's score 3+4=7 , 1 (12.5%) of them demonstrated alcian blue .None of the moderately to poorly differentiated and undifferentiated tumors showed acid mucin secretion. Number of cases showing acid mucin production is depicted in figure 10 below.

FIGURE 10-.PROPORTION OF CASES SHOWING ALCIAN BLUE POSITIVITY



Thus , it is shown that acid mucin production decreases with increase in the grade of the tumor.

DISCUSSION

DISCUSSION

TURP is Transurethral Resection of the Prostate . It is a surgery to remove the parts of the prostate gland through the penis . No incisions are required. TURP is most often done to relieve the symptoms caused by an enlarged prostate , which is mostly due to benign prostate hyperplasia and prostate adenocarcioma.

Prostate chips generated from TURP procedure from 50 cases of prostate adenocarcinoma were received in the department of Pathology , TMC. All the 50 cases were processed in a routine manner and H&E sections were studied . Also preoperative serum PSA values were obtained for all the 50 cases.

Based on the degree of glandular architectural differentiation and growth pattern in relation to the stroma , prostate adenocarcinoma cases were given different Gleason's scores ranging from 2-10.

Grading of the tumors was done using Modified Gleason Scoring system ,2005.

Tumors with Gleason score ≤ 6 were grouped as low grade tumors and those with score ≥ 7 were classified as high grade tumors. H&E sections of different grades of prostate adenocarcinoma are depicted in figures 11- 25 below.

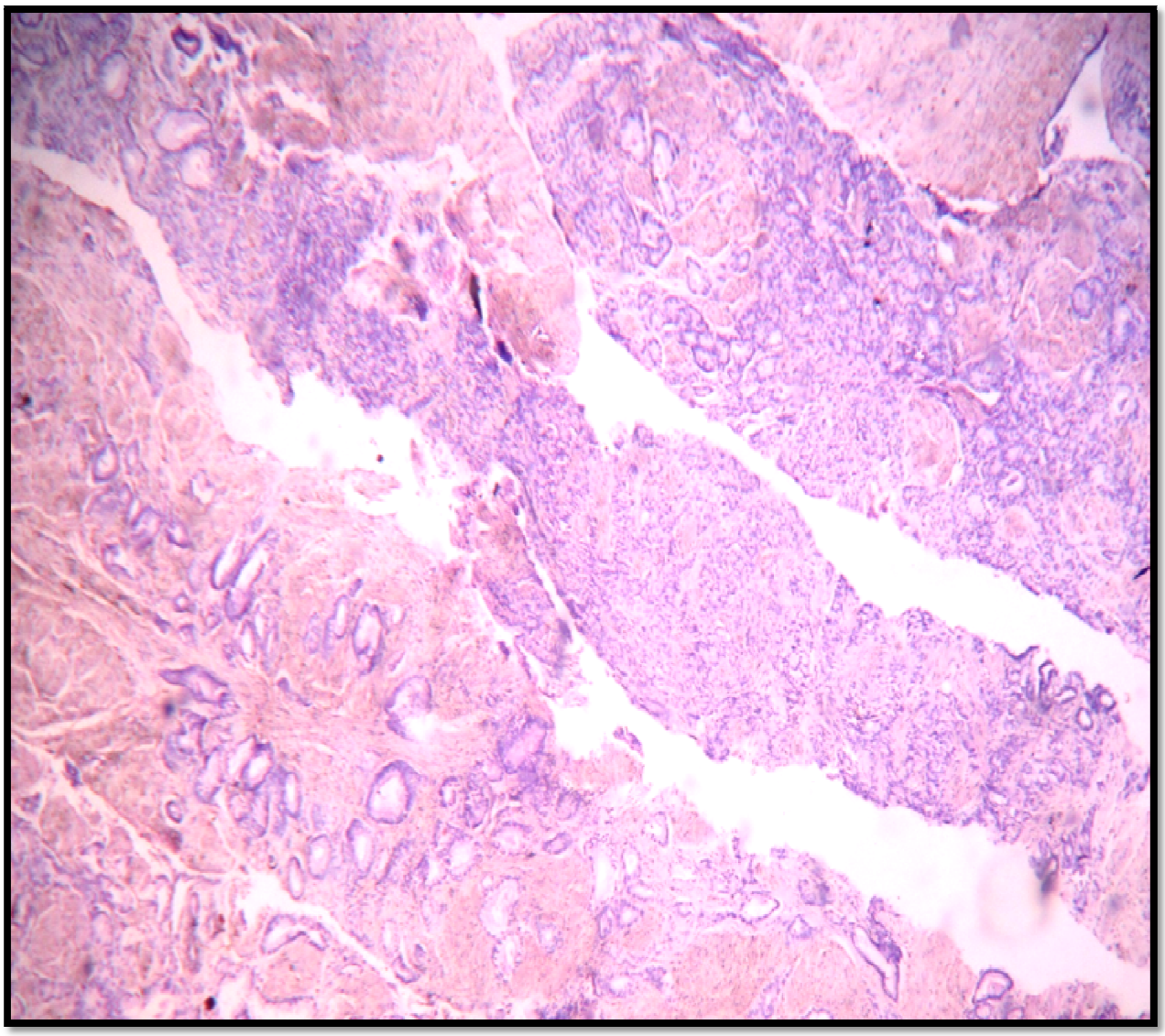


Fig 11 .Prostate adenocarcinoma - Gleason score 3+3=6 (scanner view)

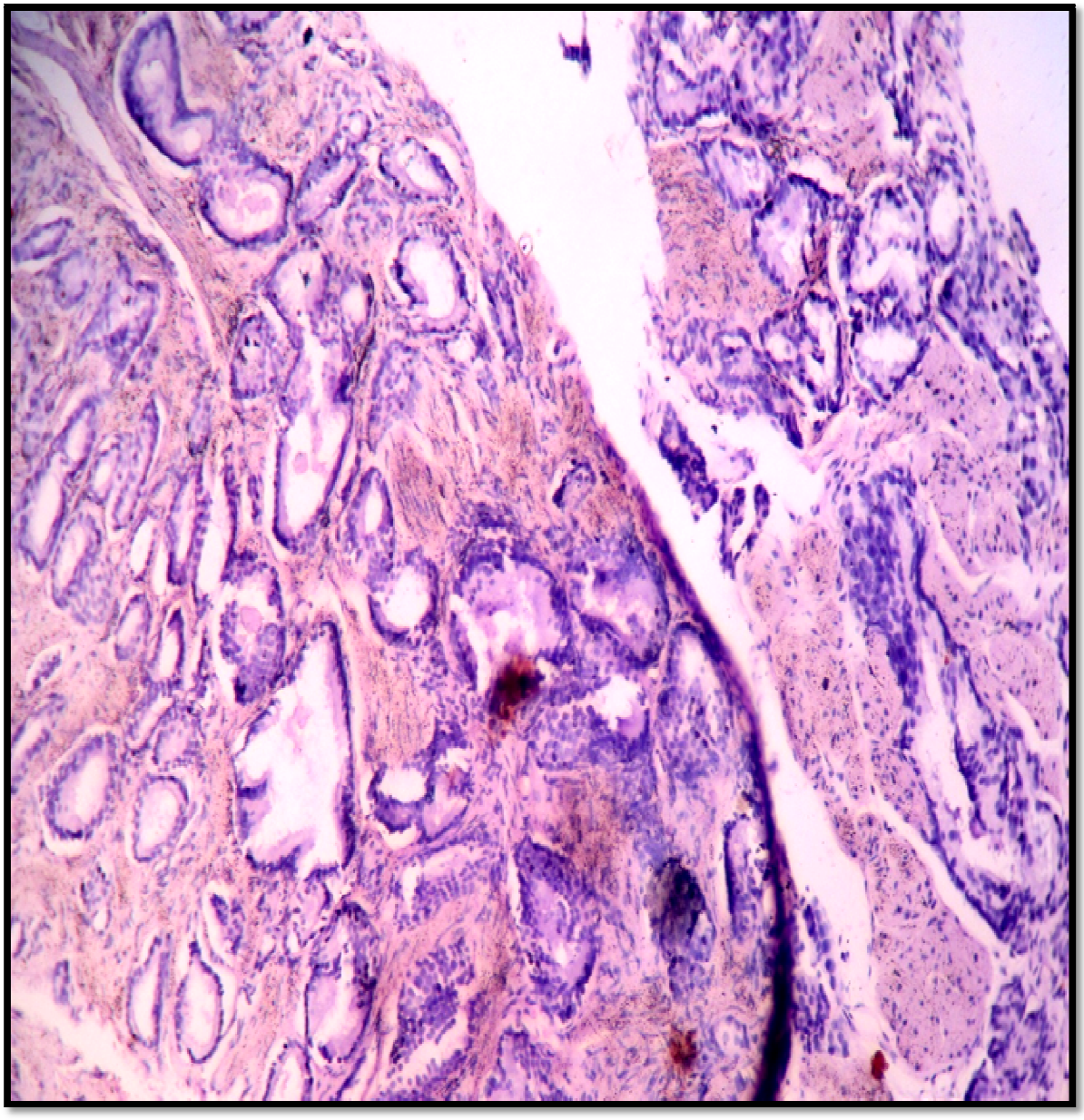


Fig 12-. Prostate adenocarcinoma - Gleason score 3+3=6 (10x)

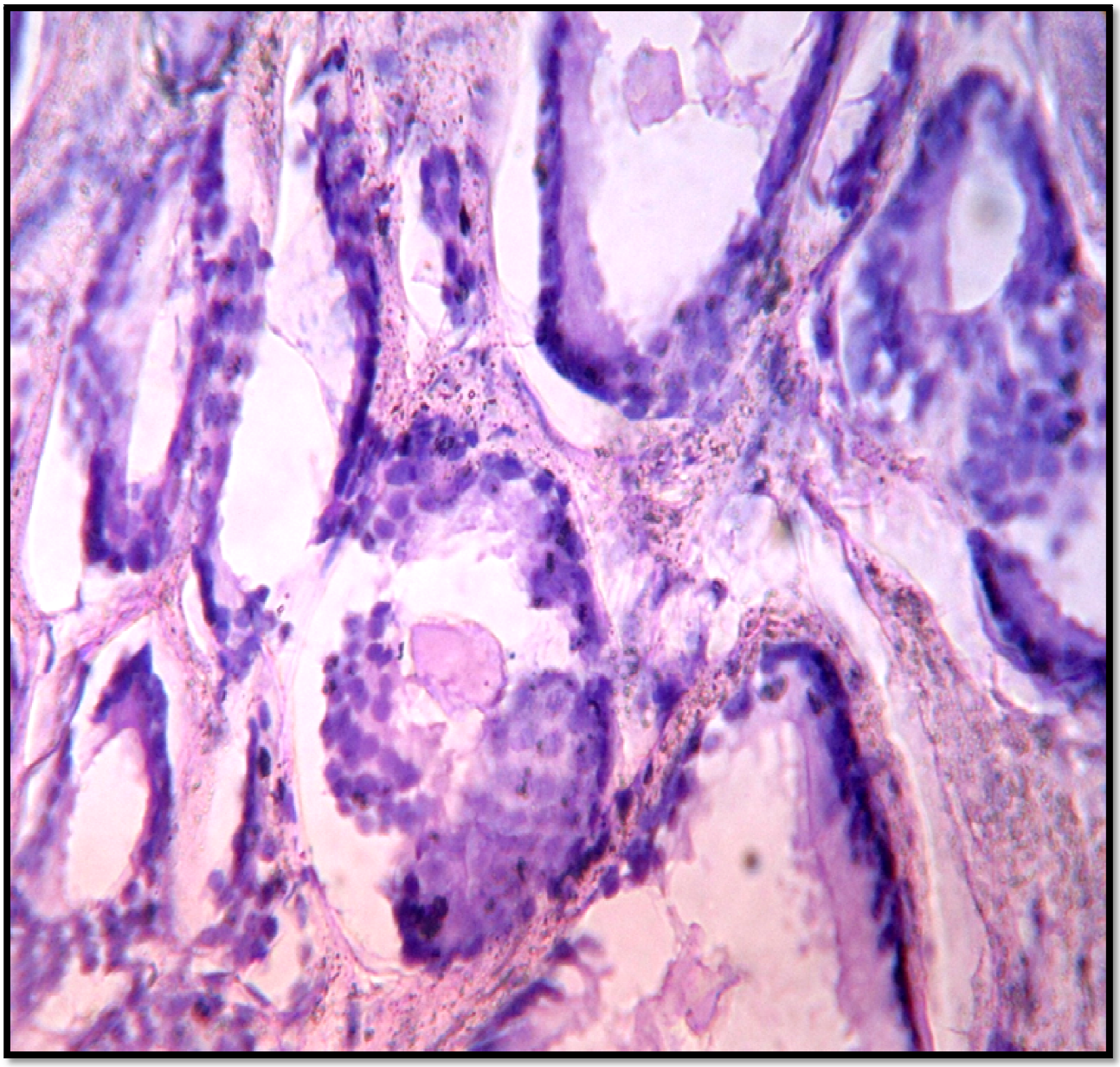


Fig13. Prostate adenocarcinoma - Gleason score 3+3=6 (40x)

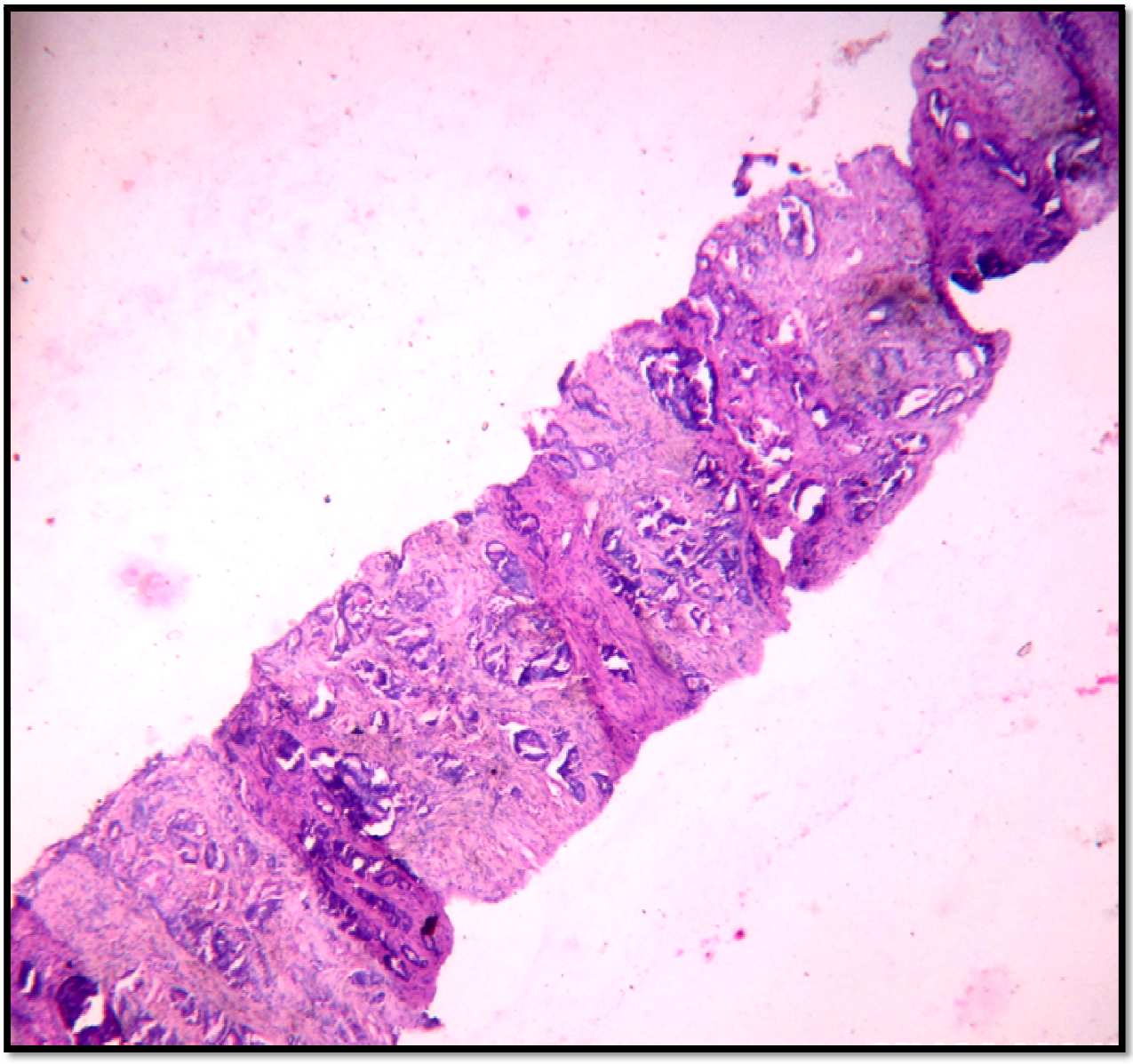


Fig14. Prostate adenocarcinoma - Gleason's score (3+2=5) -scanner view

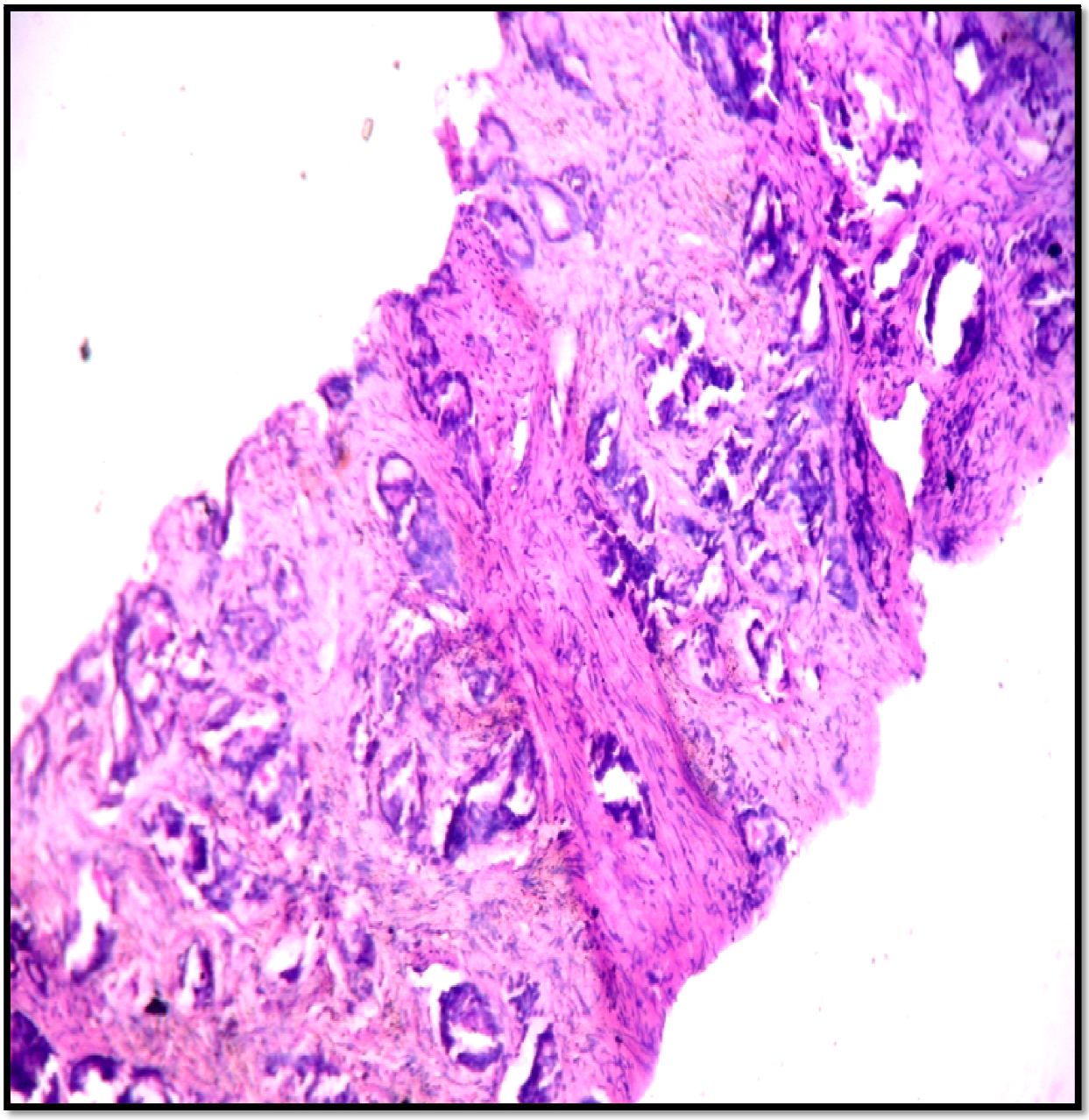


Fig 15 .Prostate adenocarcinoma - Gleason's score (3+2=5) - (10x)

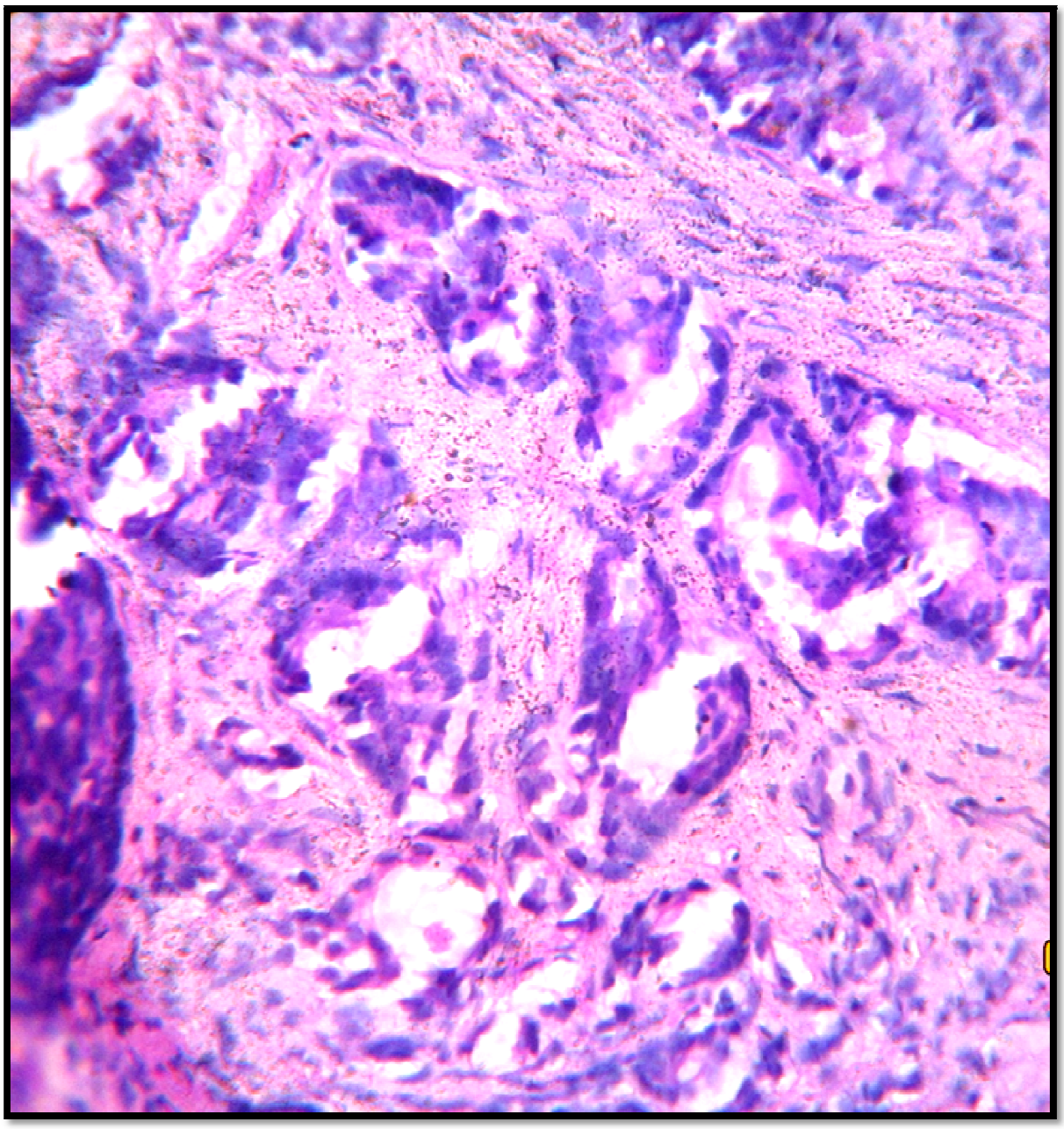


Fig16. Prostate adenocarcinoma - Gleason's score (3+2=5) - (40x)

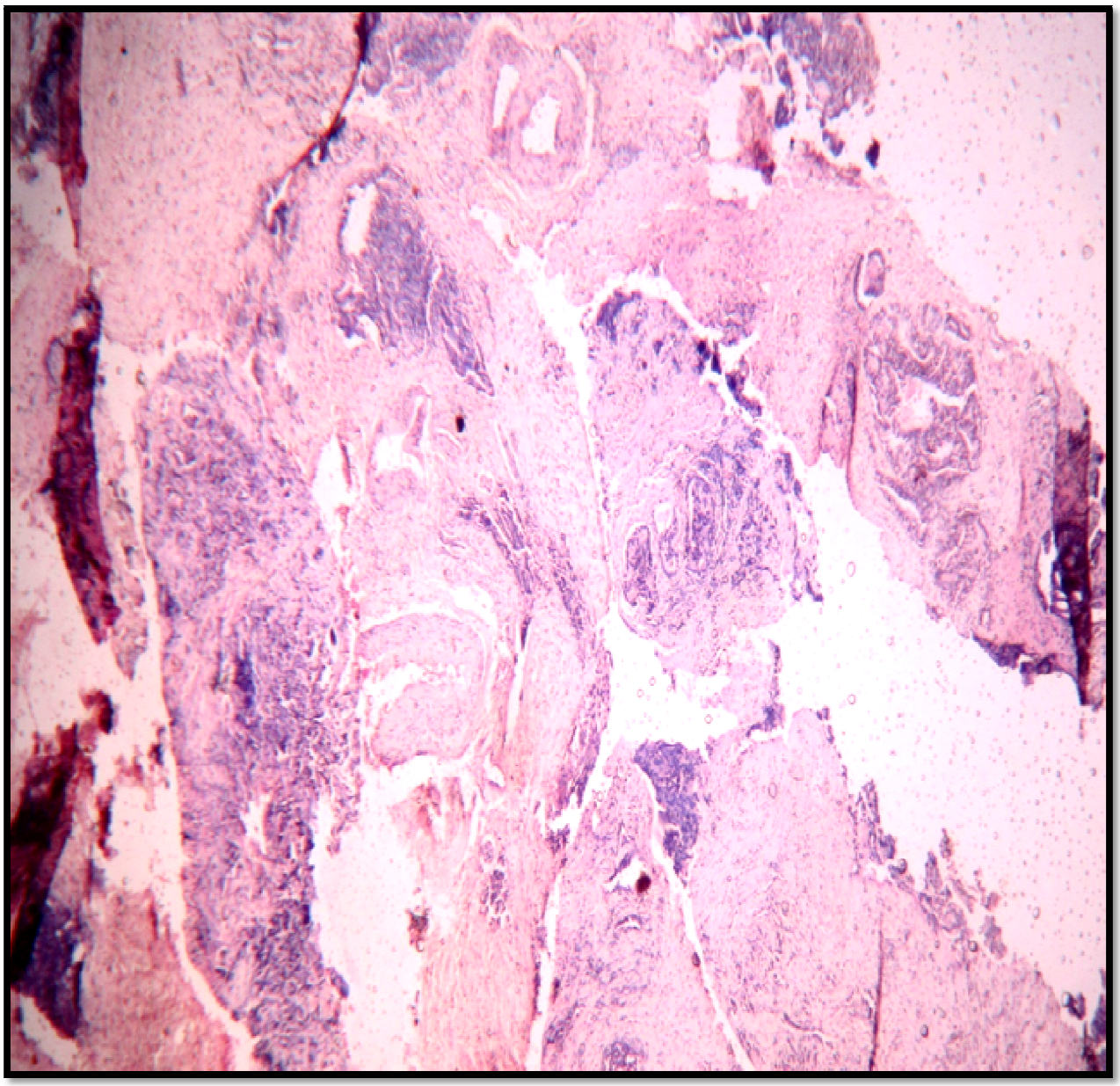


Fig 17.Prostate adenocarcinoma - Gleason's score (4+5=9) - (scanner view)

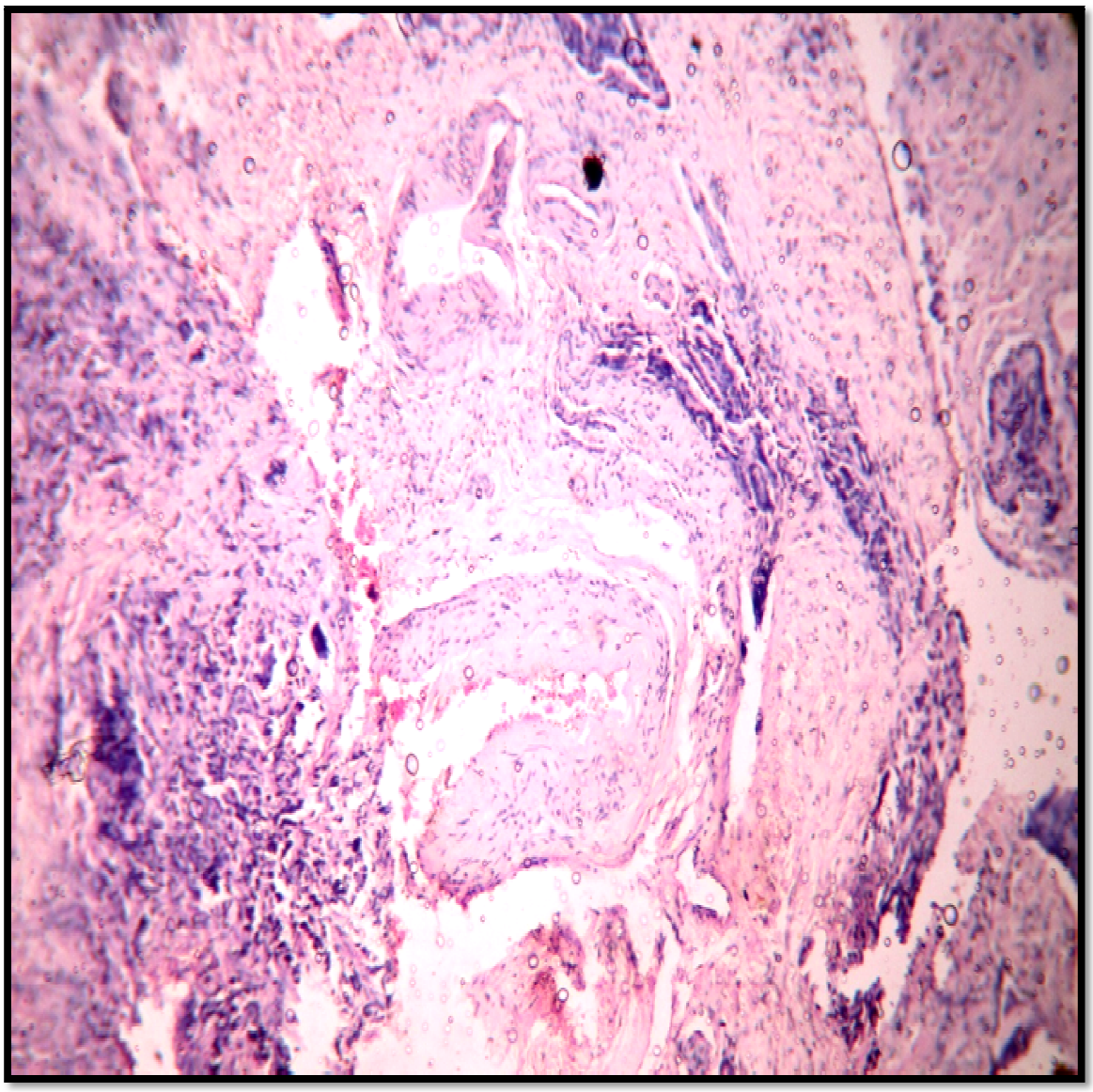


Fig18. Prostate adenocarcinoma - Gleason's score (4+5=9) - (10x)

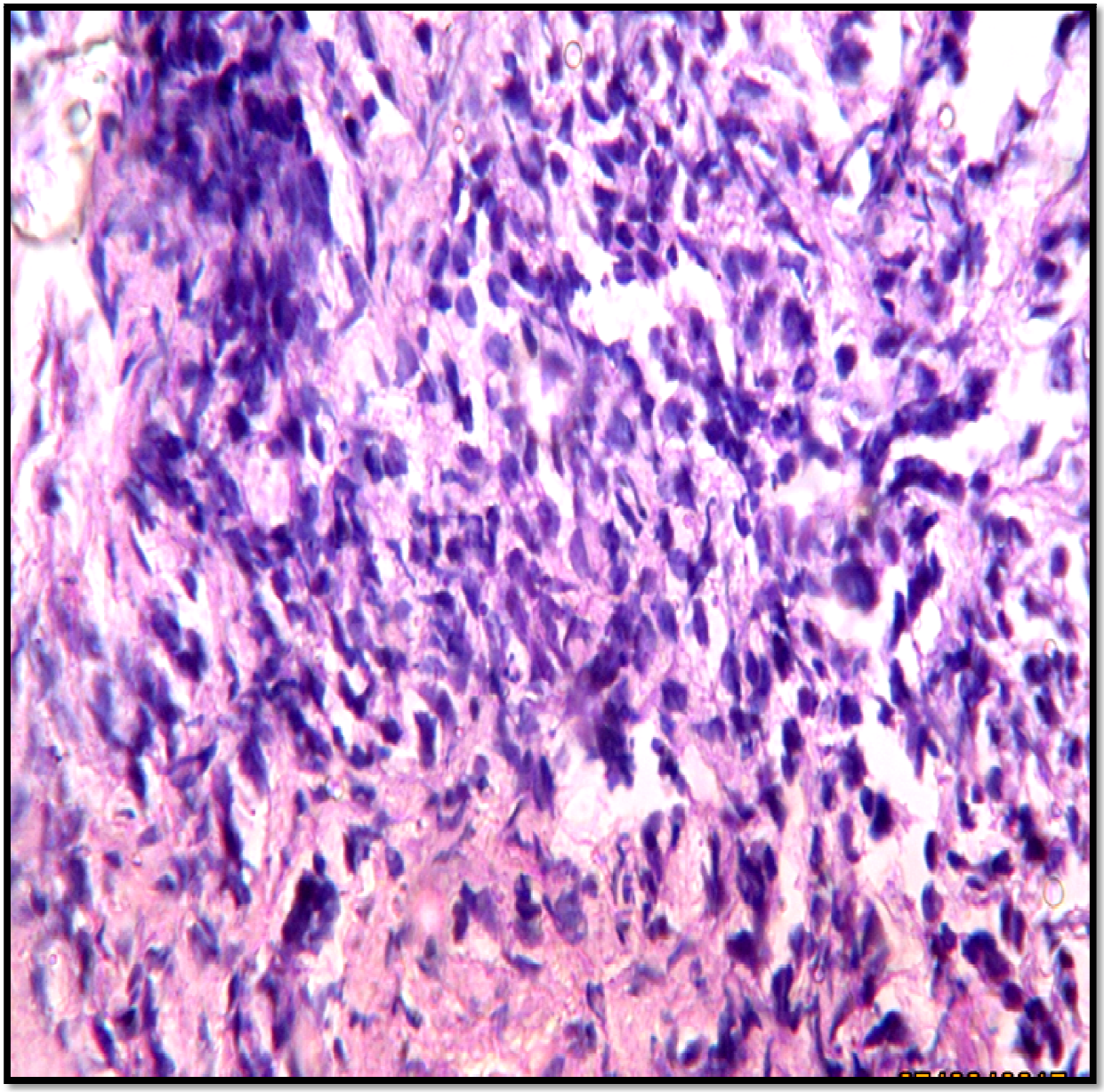


Fig 19. Prostate adenocarcinoma - Gleason's score (4+5=9) - (40x)

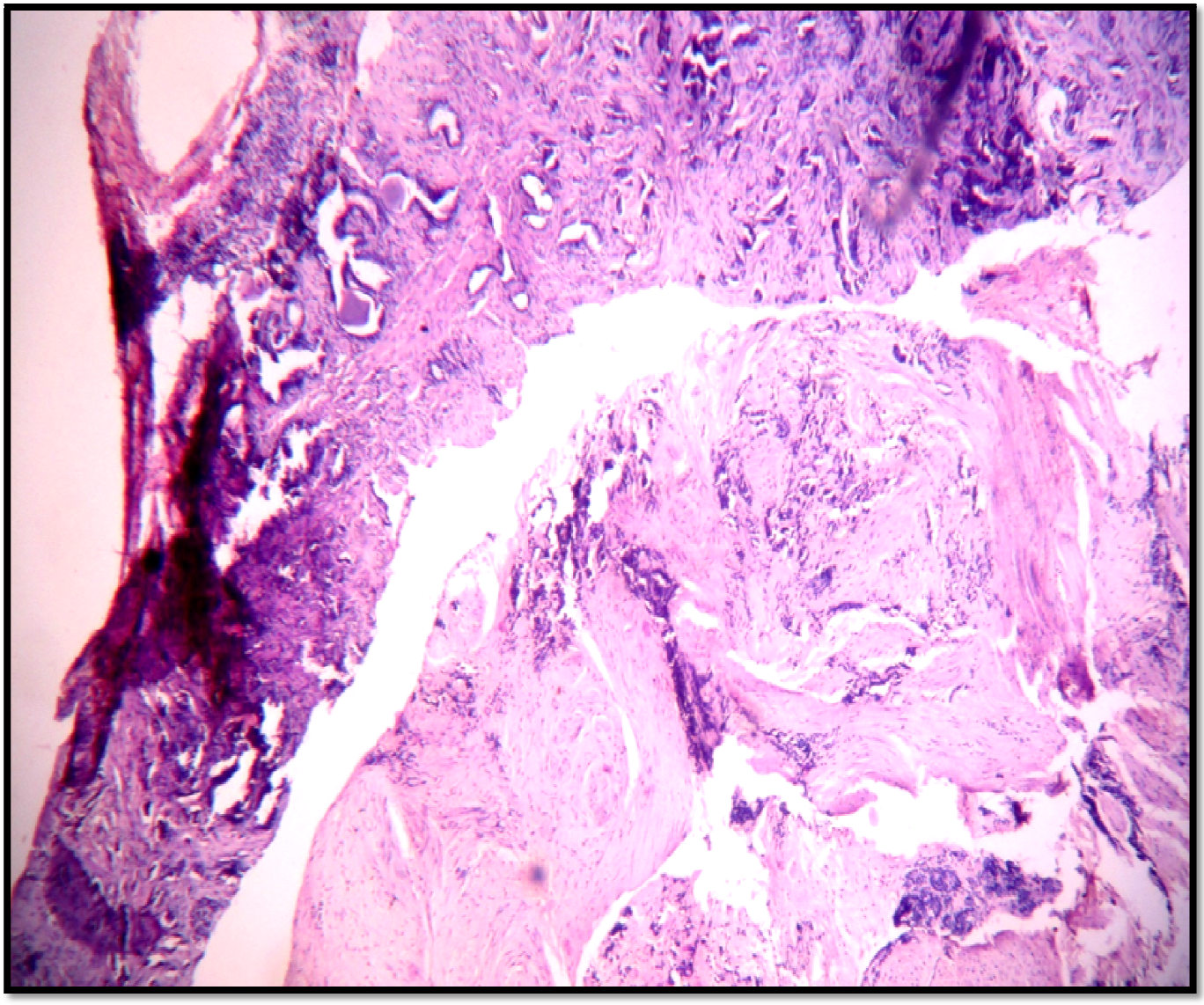


Fig 20. Prostate adenocarcinoma - Gleason's score (3+4=7) - (scanner view)

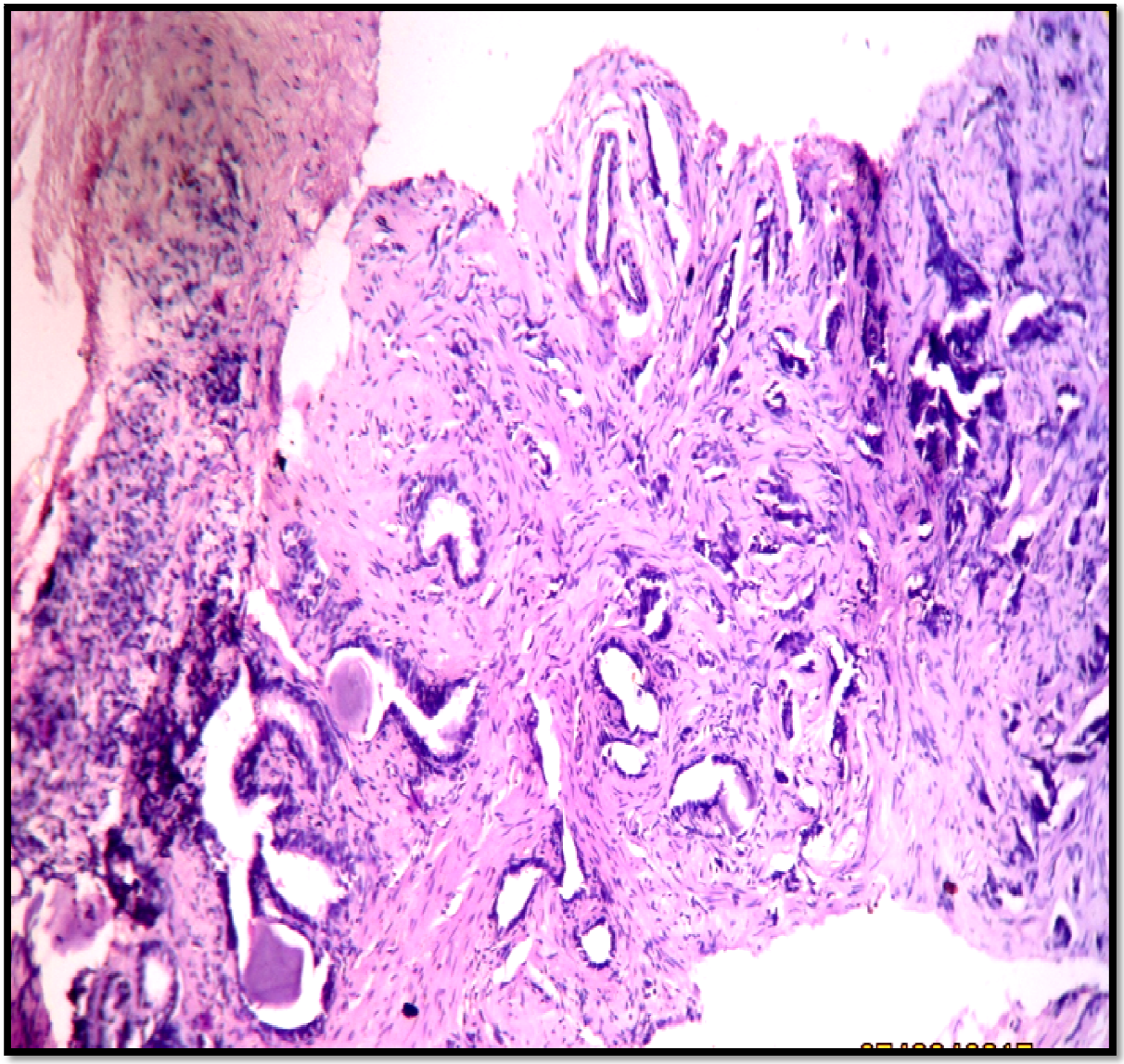


Fig .21 -Prostate adenocarcinoma - Gleason's score (3+4=7) - (10x)

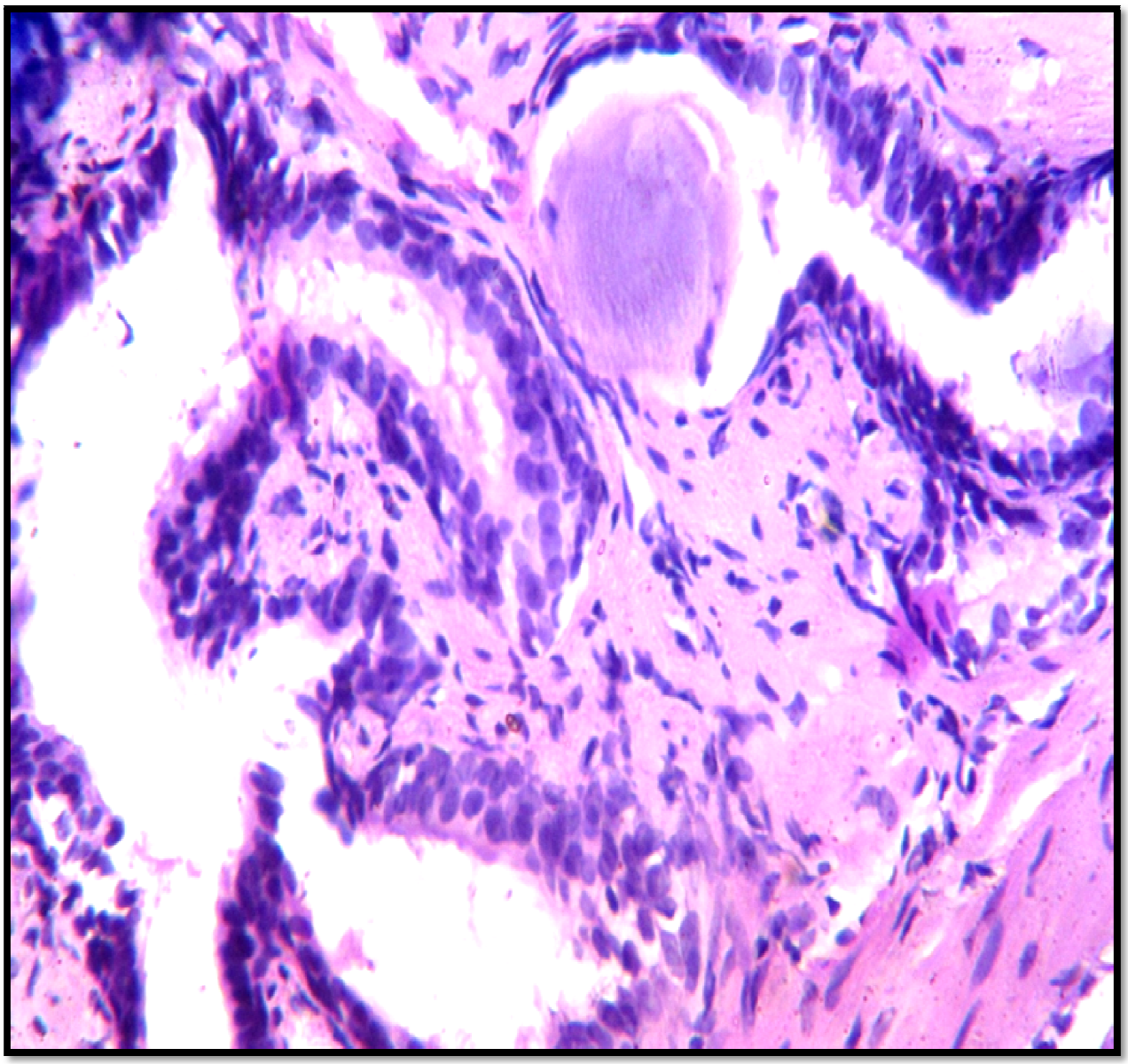


Fig. 22 - Prostate adenocarcinoma - Gleason's score (3+4=7) - (40x)

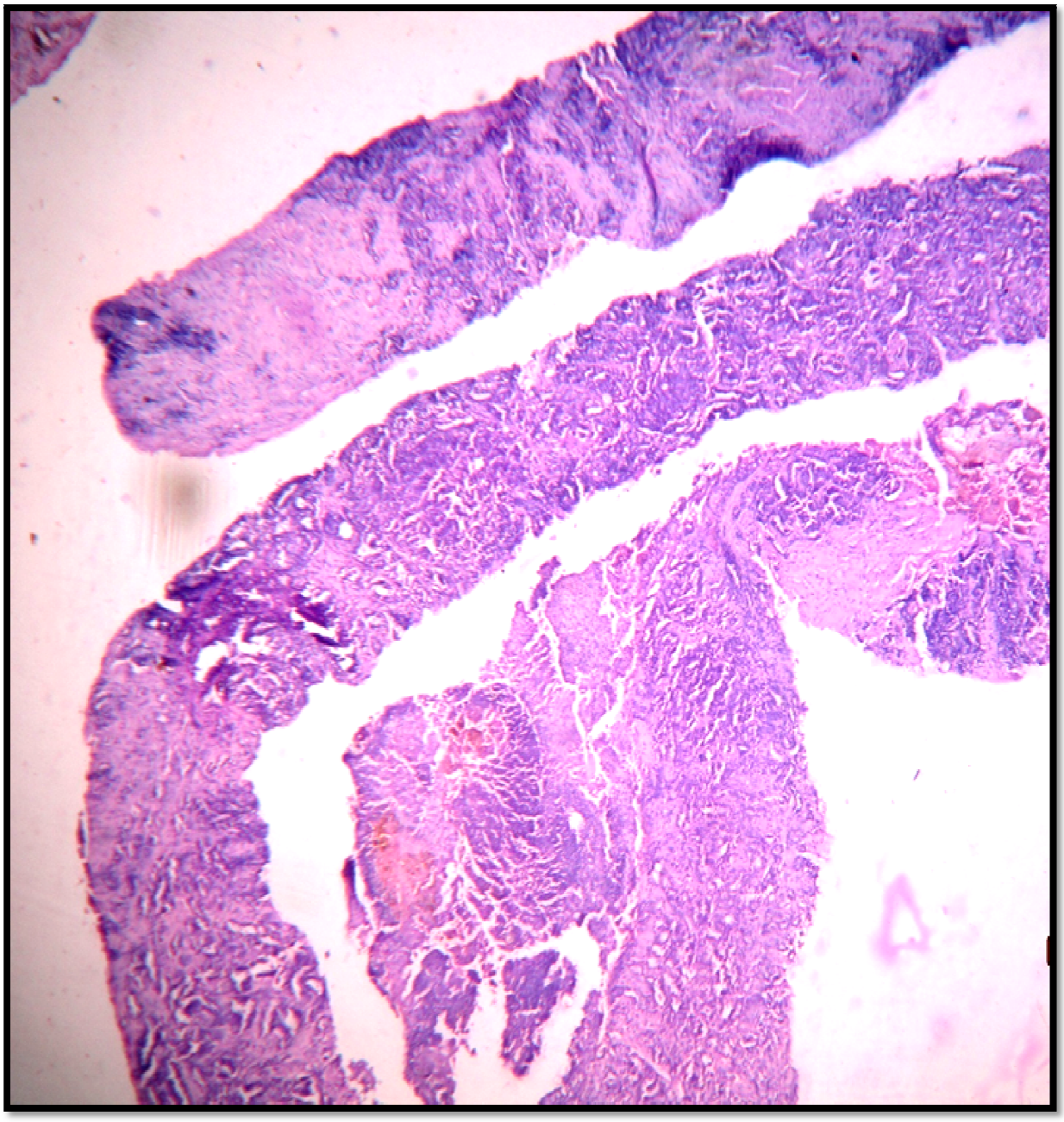


Fig.23- Prostate adenocarcinoma - Gleason's score (5+4=9) - (scanner view)

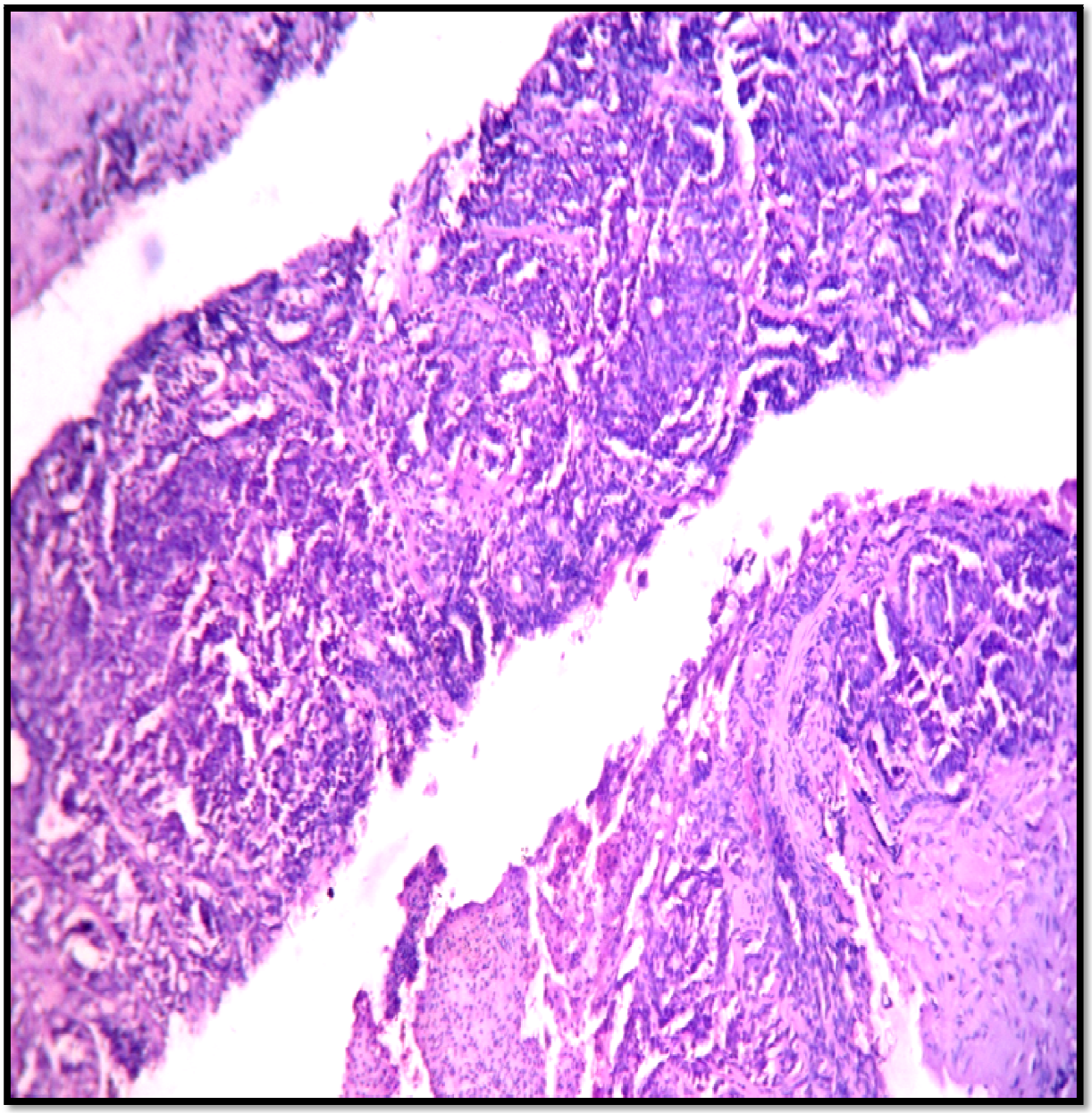


Fig.24- Prostate adenocarcinoma - Gleason's score (5+4=9) - (10x)

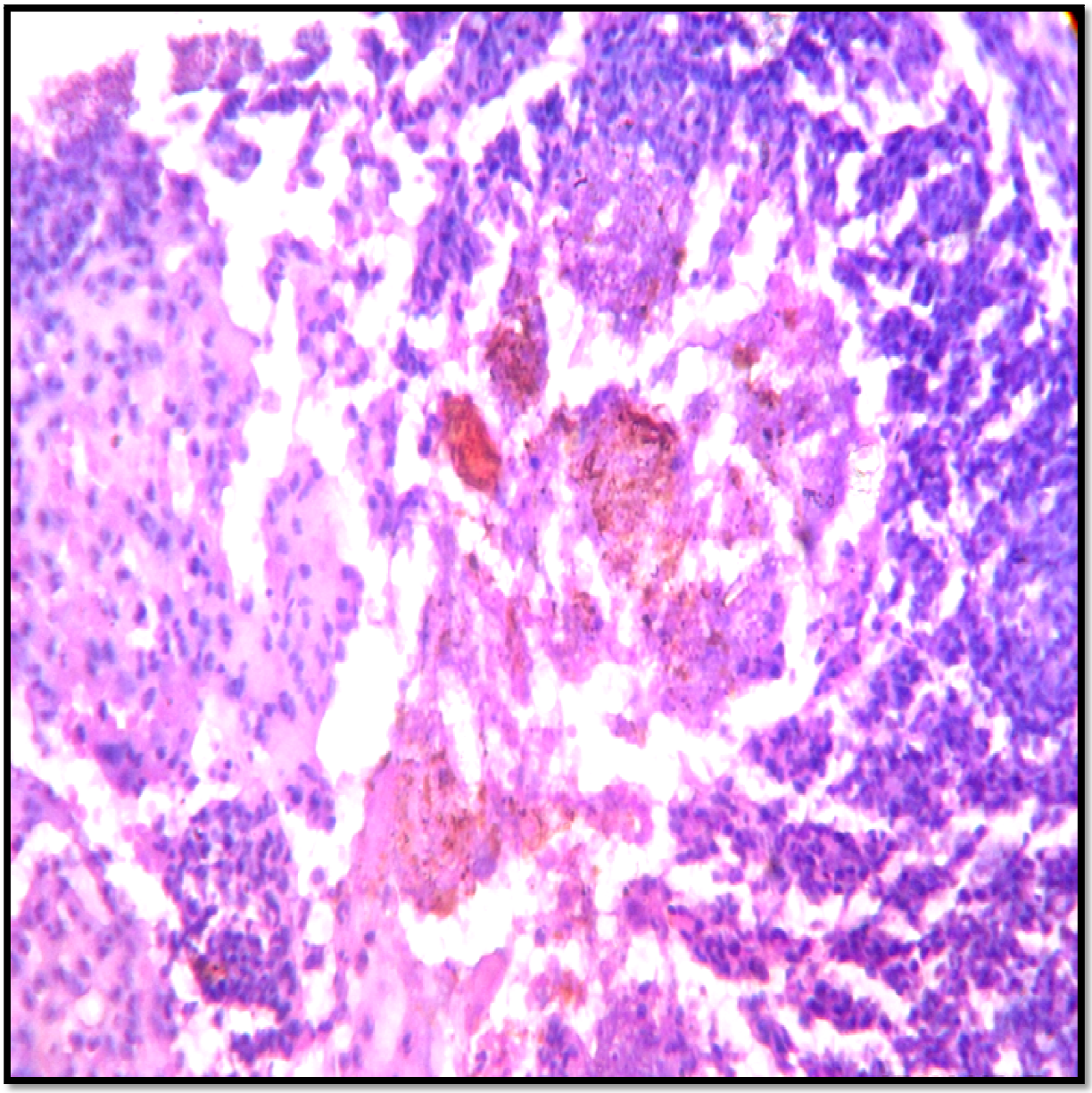


Fig. 25- Prostate adenocarcinoma - Gleason's score (5+4=9) - (40x)

AGE AT DIAGNOSIS

Prostate adenocarcinoma cases in this study were between the age group 50-80**. Peak incidence of prostate adenocarinomas was observed between the age group 70-80 (60%).**

Wein Alan J et al also stated that prostate adenocarcinoma is rare below 50 years of age and the peak incidence is between 70-74 years, with 84 % diagnosed above 65 years of age.

Ackerman et al stated that 75 % of the prostate adenocarcinomas occur in men 65 years or older.

Abbas Abul K et al also stated that the incidence of prostate adenocarcinoma increases from 20% in men in their 50s to approximately 70% in men between the ages of 70 and 80 years.

Goldblum JR et al stated that prostate adenocarcinomas are uncommon below 50 years of age and 40 times more common in men above 65 years .

Moch ,H et al , stated that worldwide 3/4 th (75%) of the prostate adenocarcinomas occur in men above 65 years of age.

Rubin R et al state that > 75 % of prostate cancer cases are between the age group of 60 -80 years.

Robert PO et al also stated that 75% of prostate adenocarcinoma cases occur in men between 60-75 years of age.

COMPARISON OF THE INCIDENCE OF PROSTATE

ADENOCARCINOMA CASES

S.NO.	AUTHORS	AGE GROUP WITH MAJORITY OF PROSTATE ADENOCARCINOMA CASES (years)	INCIDENCE OF CASES (%)
1.	Abbas AK et al	70-80	70
2.	Ackerman et al	> 65	75
3.	Wein Alan J et al	> 65	84
4.	Robert PO et al	60-75	75
5.	Rubin R et al	60-80	75
6.	Moch, H et al	> 65	75
7.	Present study	71-80	60

DISTRIBUTION OF PROSTATE ADENOCARCINOMA CASES ACCORDING TO THE LOCATION OF THE TUMOUR WITHIN THE GLAND.

Among the 50 cases studied , all the 50 cases (100%) had cancer located in the peripheral zone of the gland .

William K. Oh et al also state that the peripheral zone is the most common site in the prostate for developing prostate carcinoma.

Abbas AK et al also state that in approximately 70% of the cases , prostate carcinoma arises in the peripheral zone of the gland , classically in a posterior location.

Fletcher CDM. also stated that 68% of the carcinomas arise in peripheral zone.

Bostwick DG et al stated that 70% or more of the prostate cancers involve the peripheral zone of the prostate.

Mohan H . stated that in 95 % of cases , prostate carcinoma is located in the peripheral zone , especially the posterior lobe.

COMPARISON OF PROPORTION OF CASES INVOLVING THE PERIPHERAL ZONE OF THE PROSTATE GLAND

S.NO.	AUTHORS	PROPORTION OF CASES INSVOLVING THE PERIPHERAL ZONE
1.	Abbas AK et al	70%
2.	Fletcher CDM et al	68%
3.	Bostwick DG et al	70%
4.	Mohan H et al	95%
5.	Present study	100%

CORRELATION OF S .PSA LEVEL WITH GLEASON SCORE

A positive correlation exists between preoperative serum PSA levels and Gleason score.

Shih WJ et al conducted a study in 65 men and stated that 23 men with low Gleason score had an average preoperative serum PSA level of 23.62 and 42 men with high Gleason score (6-10) had a mean Gleason score of 134.39. Thus, they showed that there is a strong correlation between Gleason score and serum PSA level.[29]

Issac AS et al conducted a study in 16 prostate adenocarcinoma cases . 4/16 cases with well differentiated adenocarcinomas had low serum PSA levels. 3/4 well differentiated adenocarcinoma cases had normal serum PSA levels and the remaining 1/4 cases of well differentiated tumors had serum PSA level 8.8 ng/mL (borderline PSA level). 12 cases had high Gleason score ranging between 6-9. All these cases had serum PSA level > 100 ng/mL with a mean of 208 . [30]

STUDY BY ISSAC et al

S.NO	SERUM PSA LEVEL	GLEASON SCORE
1.	2.9	2
2.	0.9	3
3.	8.8	3
4.	2	4
5.	122	6
6.	145	6
7.	160	6
8.	105	8
9.	116	8
10.	136	8
11.	120	8
12.	256	8
13.	850.7	8
14.	165	9
15.	178	9
16.	152	9

In the present study which includes 50 cases , mean serum PSA level was calculated for cases with low and high Gleason score. Low Gleason score includes cases with score between 2-5 and high Gleason score includes cases with a score between 6-10 as the above two studies. There are 23 cases and 27 cases with low and high Gleason scores respectively.

Mean S. PSA level among cases with low Gleason score = 27.9 ng/mL

Mean S. PSA level among cases with high Gleason score = 139.25 ng/mL

S.NO.	AUTHORS	GLEASON SCORE	NO. OF CASES	MEAN S.PSA LEVEL (ng/mL)
1.	Shih WJ et al	High (6-10)	42/65	134.39
2.	Isacc et al	High (6-10)	12/16	208
3.	Present study	High (6-10)	27/50	139.25

S.NO.	AUTHORS	GLEASON SCORE	NO. OF CASES	MEAN S.PSA LEVEL (ng/mL)
1.	Shih WJ et al	Low (2-5)	23/65	23.62
2.	Isacc et al	Low (2-5)	1/4	8.8
3.	Present study	Low (2-5)	23/50	27.9

Thus, there is a correlation between Gleason score and serum PSA levels. The serum PSA level increases as the score increases.

CORRELATION OF CYCLIN D1 POSITIVITY WITH GLEASON SCORE

Pereira R.A. et al conducted a study to investigate the relationship between cyclin D1 expression and clinicopathological parameters in patients with prostate cancer. IHC was done in 85 cases using cyclin D1 antibody. Nuclear immunostaining was measured and the results were given based on the percentage of positive tumor cells. Cyclin D1 staining was positive in 64/ 85 cases (75.4%) and negative in 21 /85 cases (24.7%) of the cases. Among the high grade tumors having a Gleason score ≥ 7 , 86% demonstrated cyclin D1 immunostaining. Mean expression of cyclin D1 in high grade Gleason score group was 39.6% and was 26.9% in the low Gleason score group. [48]

In the present study , IHC was done on all 50 cases. Nuclear staining was measured, and those tumors in which > 5% of the tumor cells were stained with cyclin D1 were considered to be positive and those where <5% tumor cells were stained with cyclin D1 were considered to be negative for cyclin D1.

24/50 (48%) cases were positive for cyclin D 1 .

Among the 16 high grade tumors (Gleason score ≥7) in this study , all 16 cases (100%) showed cyclin D1 positivity .

In this study only 8/34 (23.5%) low grade tumors showed cyclin D1 positivity . Mean expression of cyclin D1 in high and low grade tumors were 35% and 16% respectively . This is shown in figure 26.

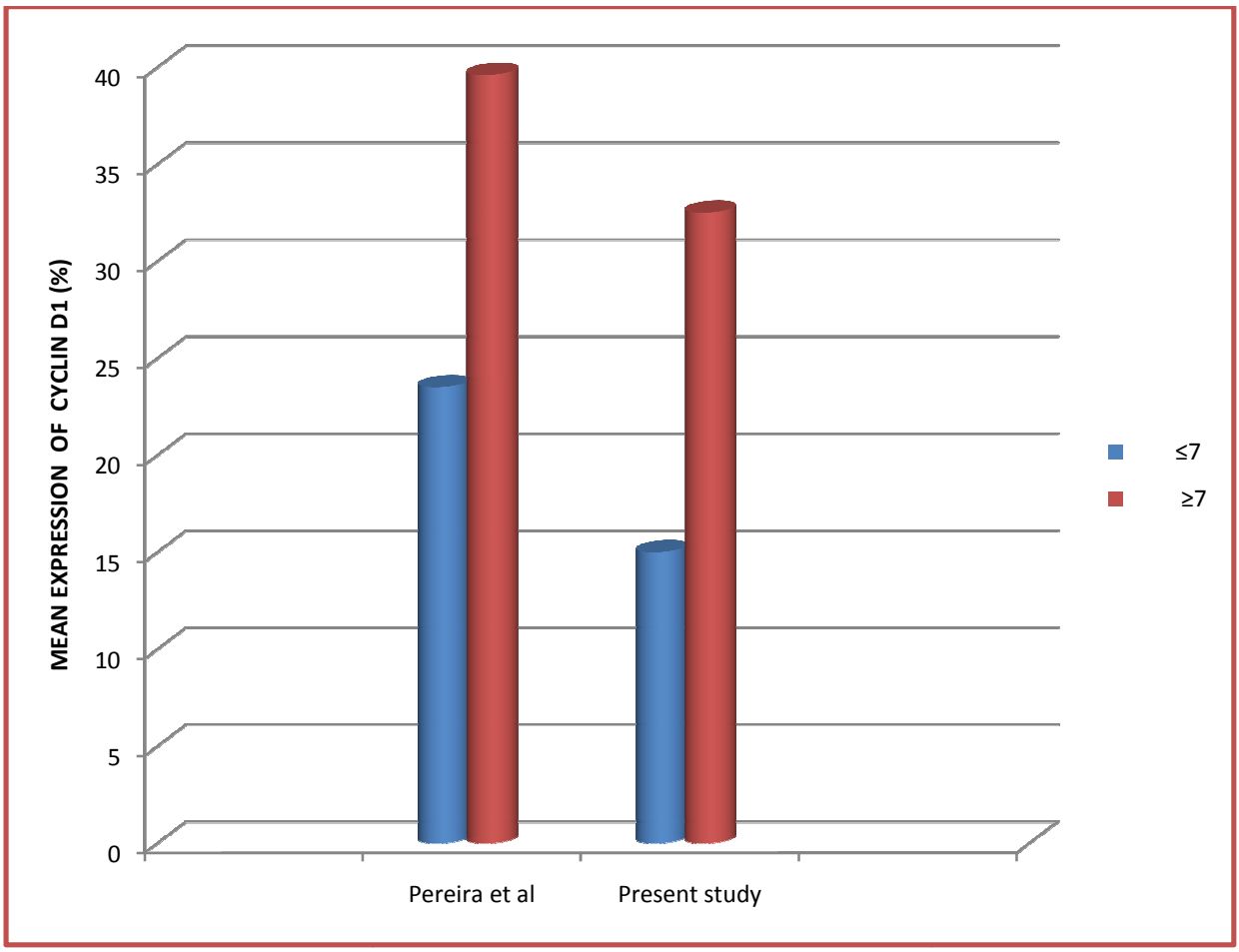
S.NO.	AUTHORS	GLEASON SCORE	% OF HIGH GRADE CASES SHOWING CYCLIN D1 +	MEAN EXPRESSION OF CYCLIN D1%
1.	Pereira R.A. et al	≥ 7 (high grade)	86%	39.6%
2.	Present	≥ 7 (high grade)	100%	35%

S.NO.	AUTHORS	GLEASON SCORE	% OF LOW GRADE CASES SHOWING CYCLIN D1 +	MEAN EXPRESSION OF CYCLIN D1%
1.	Pereira R.A. et al	≤7 (Low grade)	21%	26.9%
2.	Present	≤ 7 (low grade)	23.5%	16%

Cyclin D1	High grade tumors	Low grade tumors	Total	
+	16	8	24	p < 0.001
-	0	26	26	
Total	16	34	50	

Thus, the present study shows that nuclear expression of cyclin D1 is higher in high grade tumors than the low grade cases and also more number of high grade tumors show cyclin D1 expression than low grade ones. A significant correlation (P- value <0.001) is found between nuclear expression of cyclin D1 and grade of the tumor. Figures 27 to 34 show cyclin D1 expression by high grade tumors. Figures 35 to 38 show low grade prostate adenocarcinomas expressing cyclin D1. Figures 39 to 42 show low grade tumors with lack of cyclin D1 expression.

Fig 26 – COMPARISON OF MEAN EXPRESSION OF CYCLIN D1 BETWEEN LOW AND HIGH GRADE TUMORS



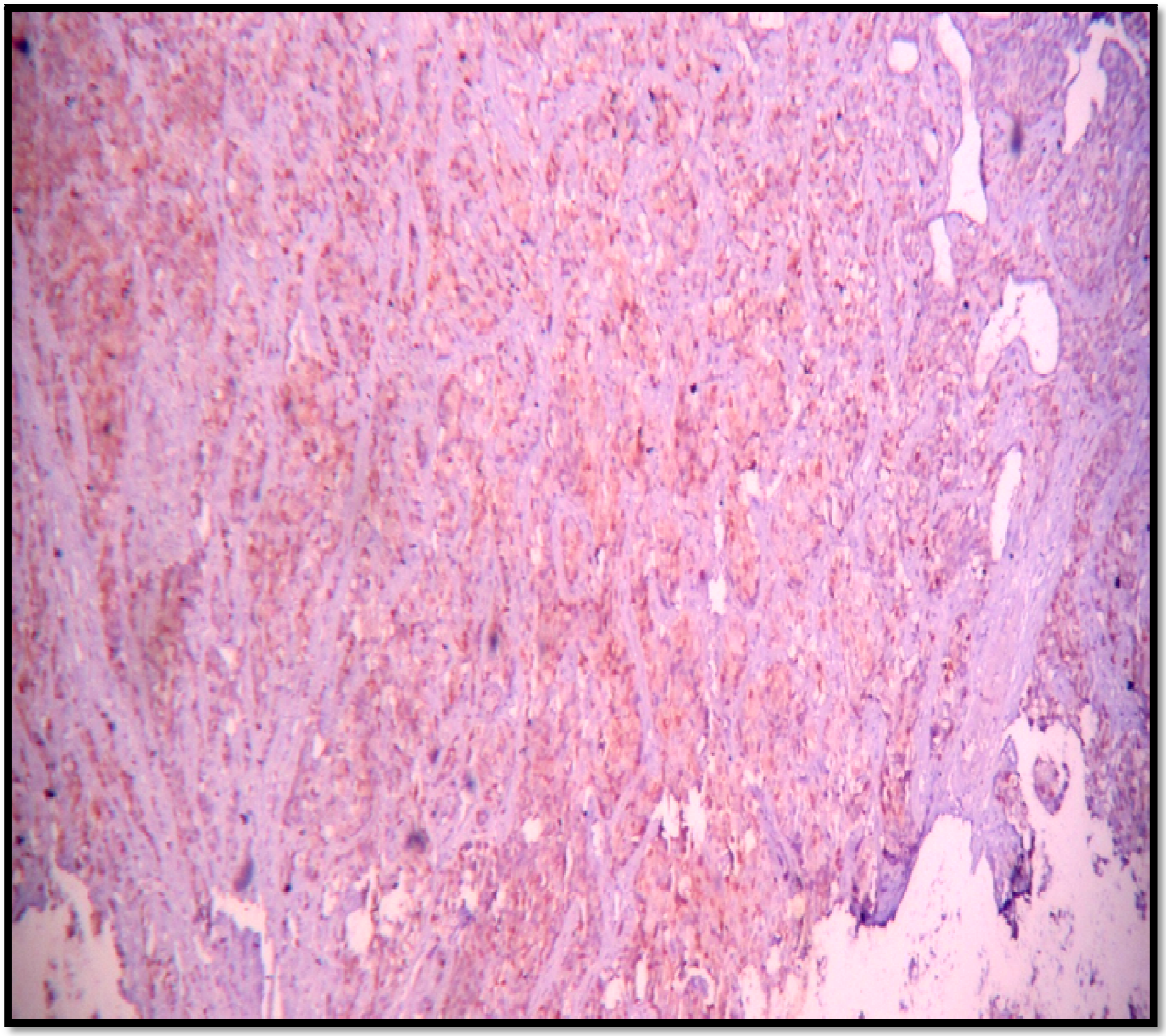


Fig 27- Nuclear expression of cyclin D1 in high grade prostate carcinoma (4+5=9) - 10x.

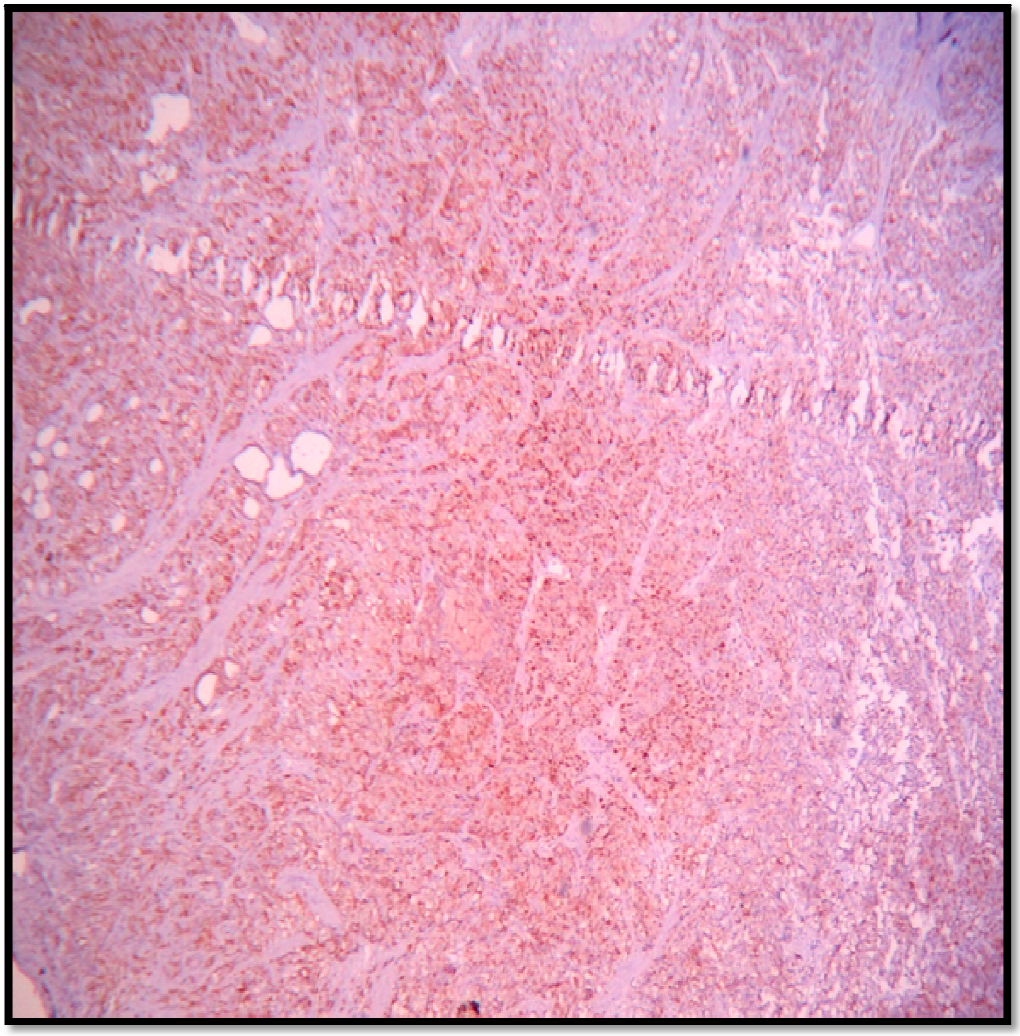


Fig.28-Nuclear expression of cyclin D1 in high grade prostate carcinoma (4+5=9) - 40x.

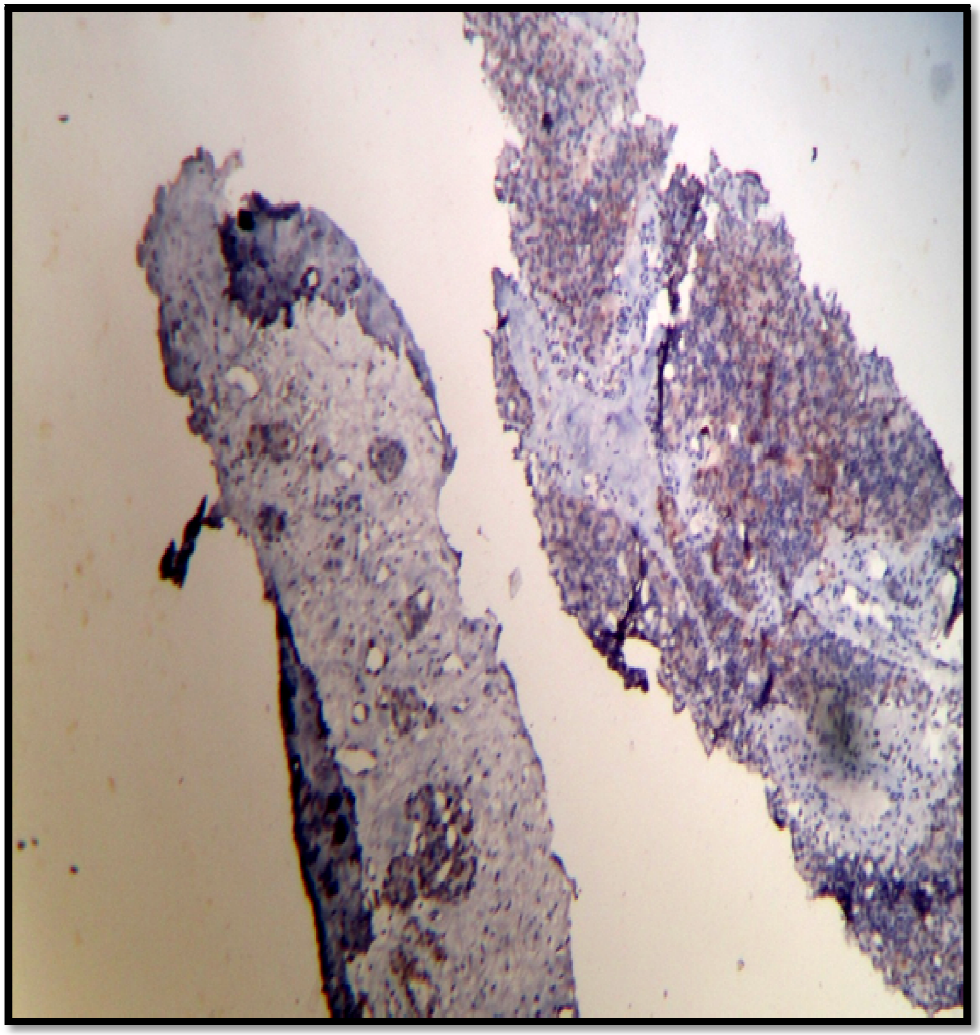


Fig .29 -Nuclear expression of cyclin D1 in high grade prostate carcinoma (4+5=9) - 10x.

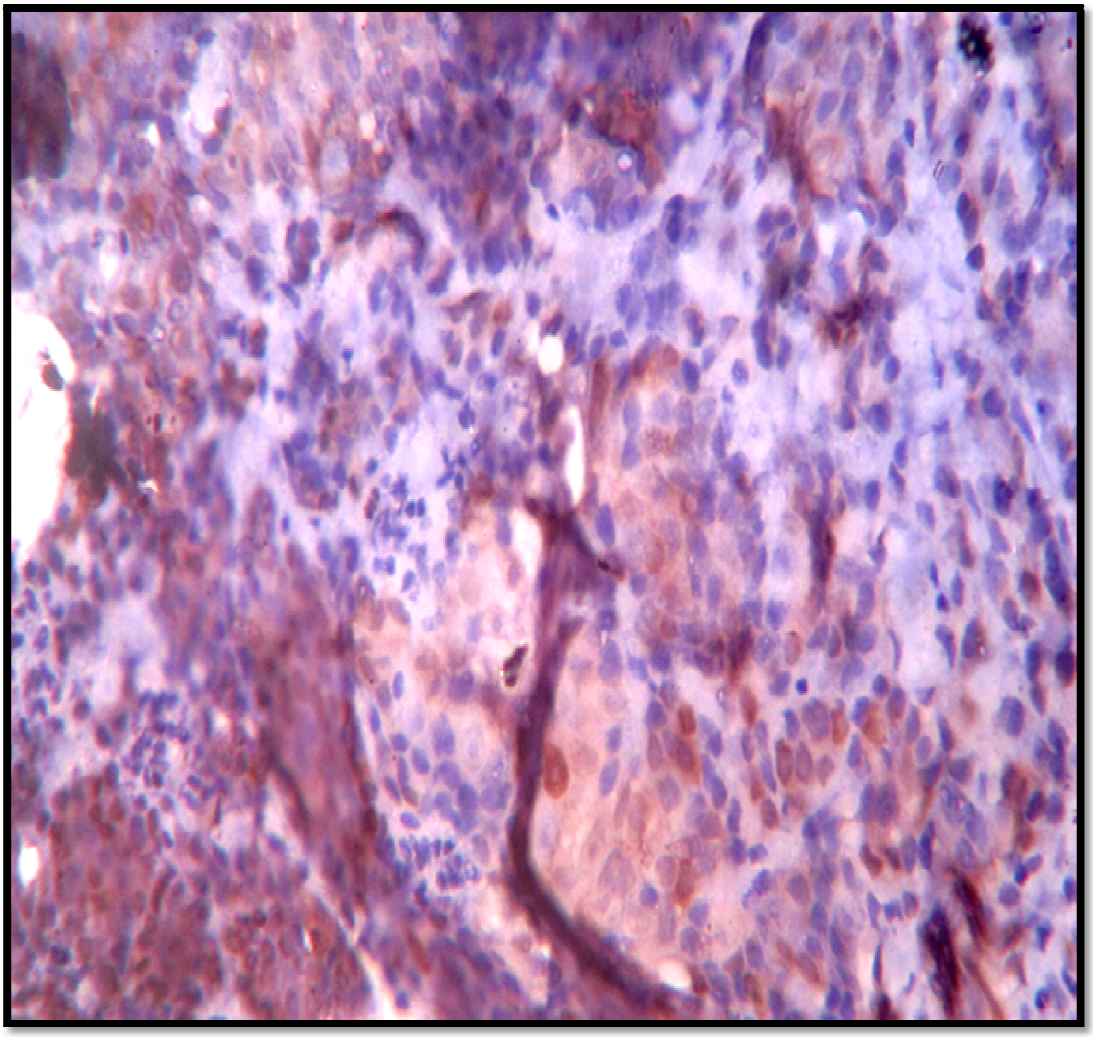


Fig. 30-Nuclear expression of cyclin D1 in high grade prostate carcinoma (4+5=9) - 40x.

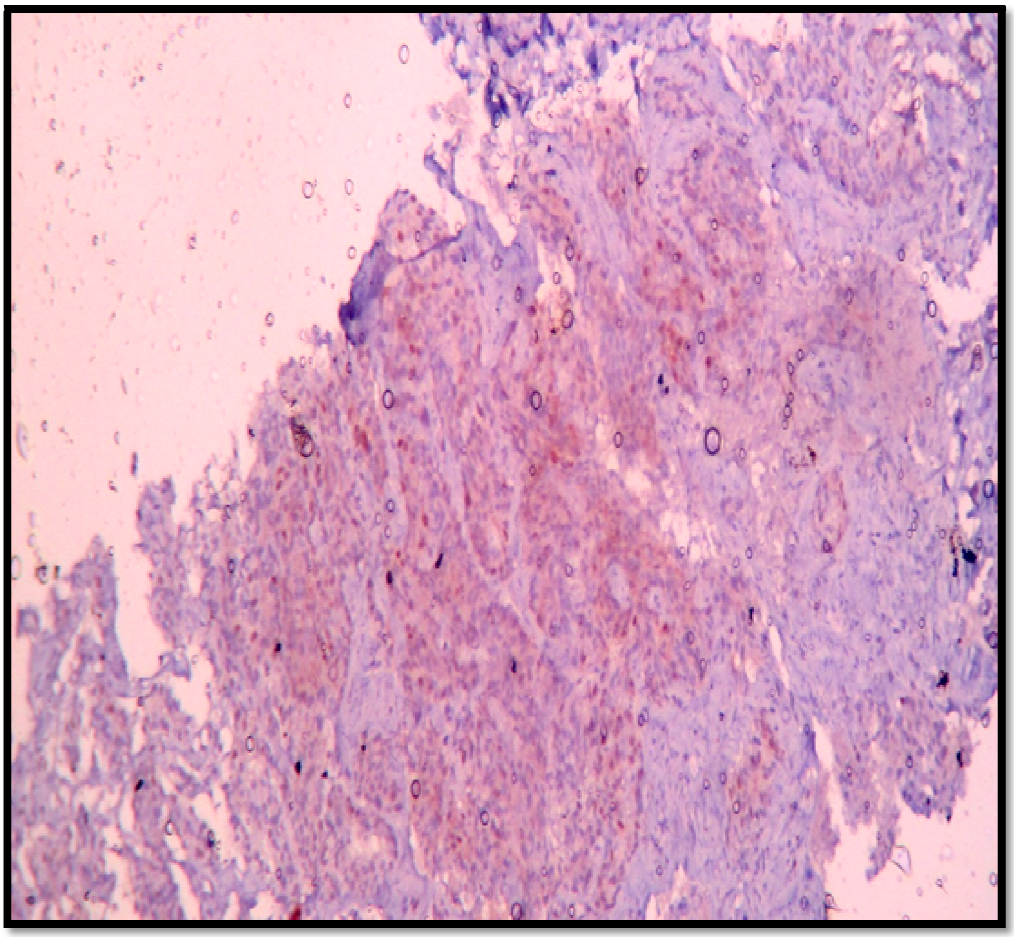


Fig .31- Nuclear expression of cyclin D1 in high grade prostate carcinoma (5+4=9) - 10x.

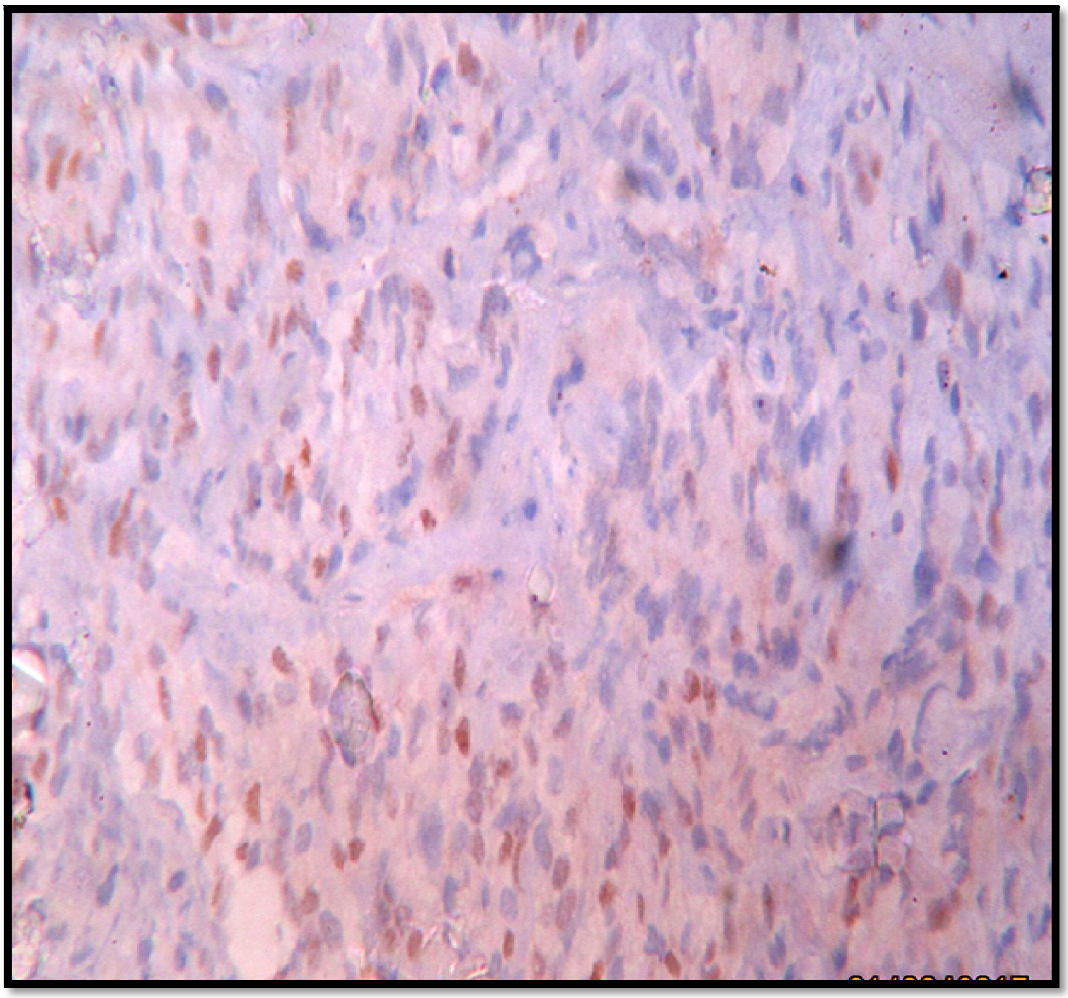


Fig .32- Nuclear expression of cyclin D1 in high grade prostate carcinoma (5+4=9) - 40x.

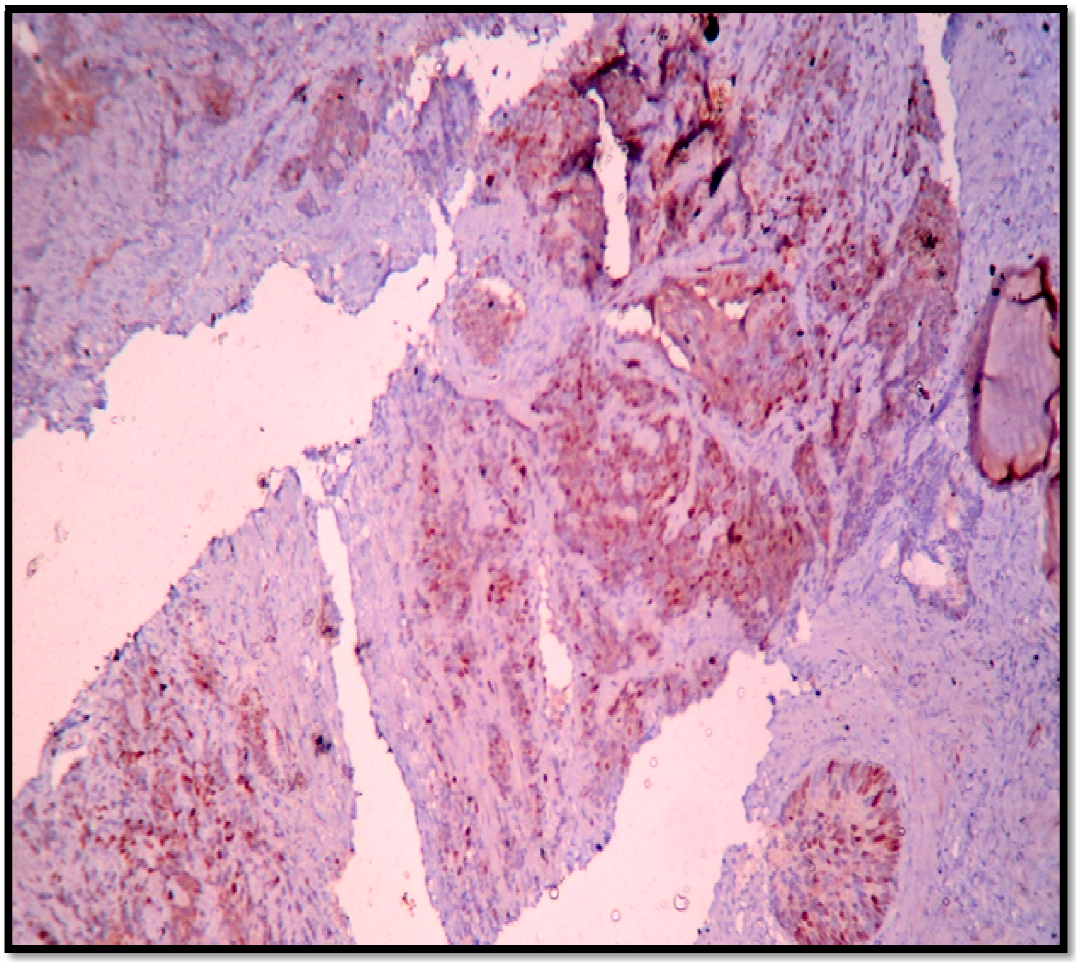


Fig.33 -Nuclear expression of cyclin D1 in high grade prostate carcinoma (3+4=7) - 10x.

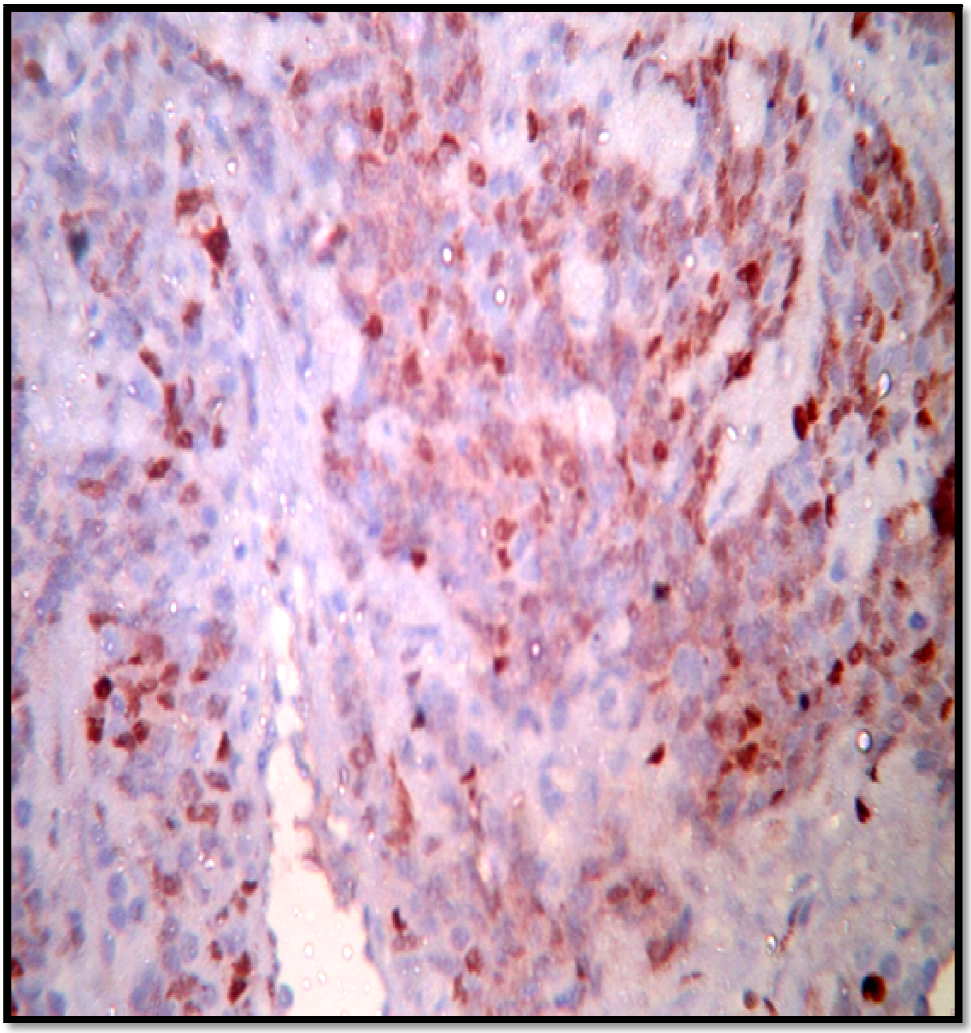


Fig .34- Nuclear expression of cyclin D1 in high grade prostate carcinoma (3+4=7) -40x.

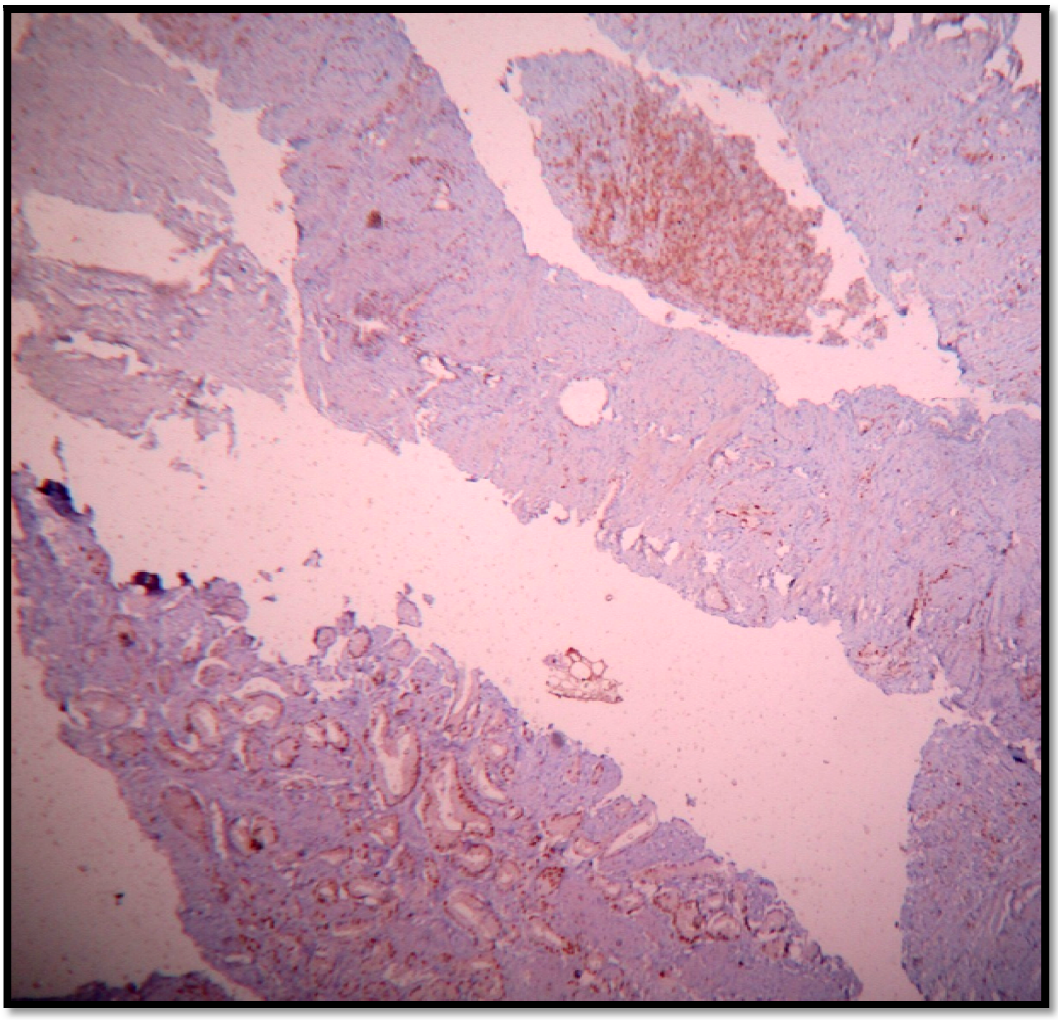


Fig .35- Nuclear expression of cyclin D1 in low grade prostate carcinoma (3+3=6) -10x.

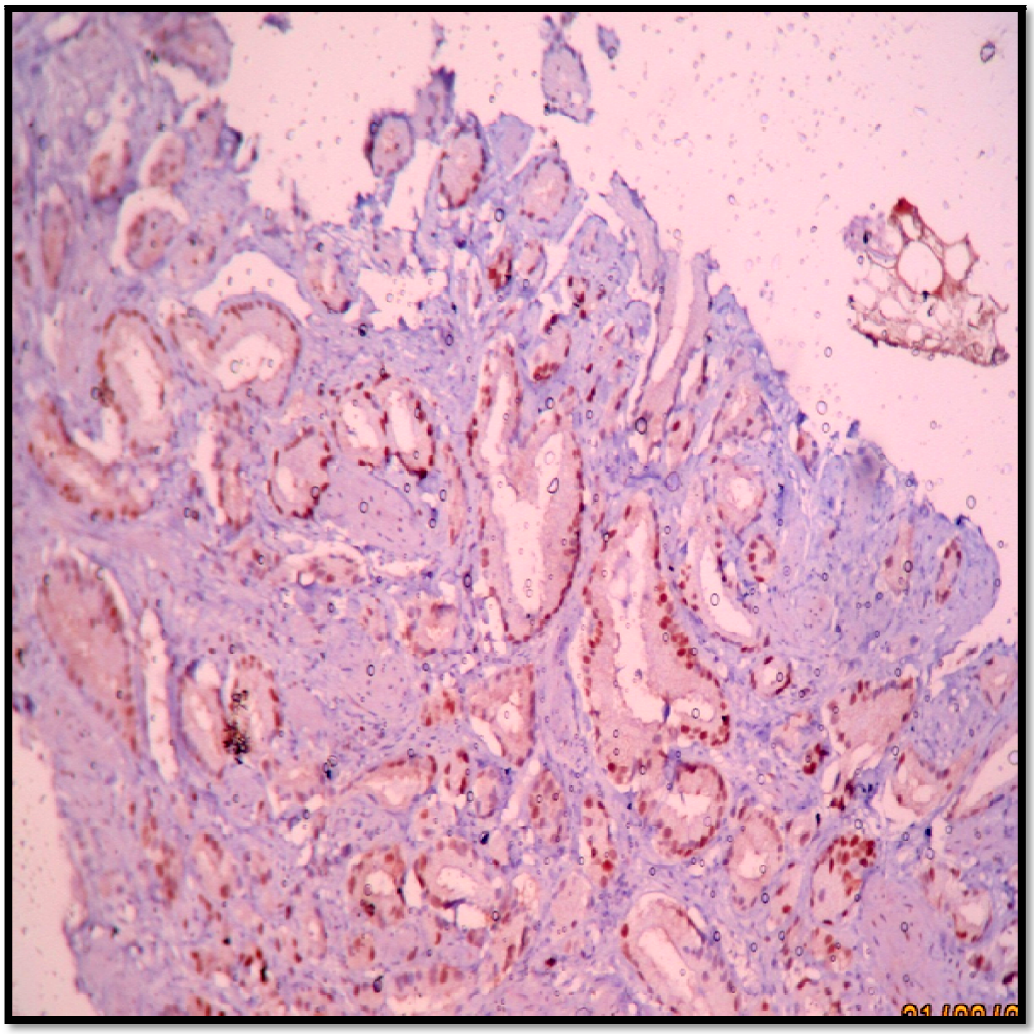


Fig .36- Nuclear expression of cyclin D1 in low grade prostate carcinoma (3+3=6) -40x.

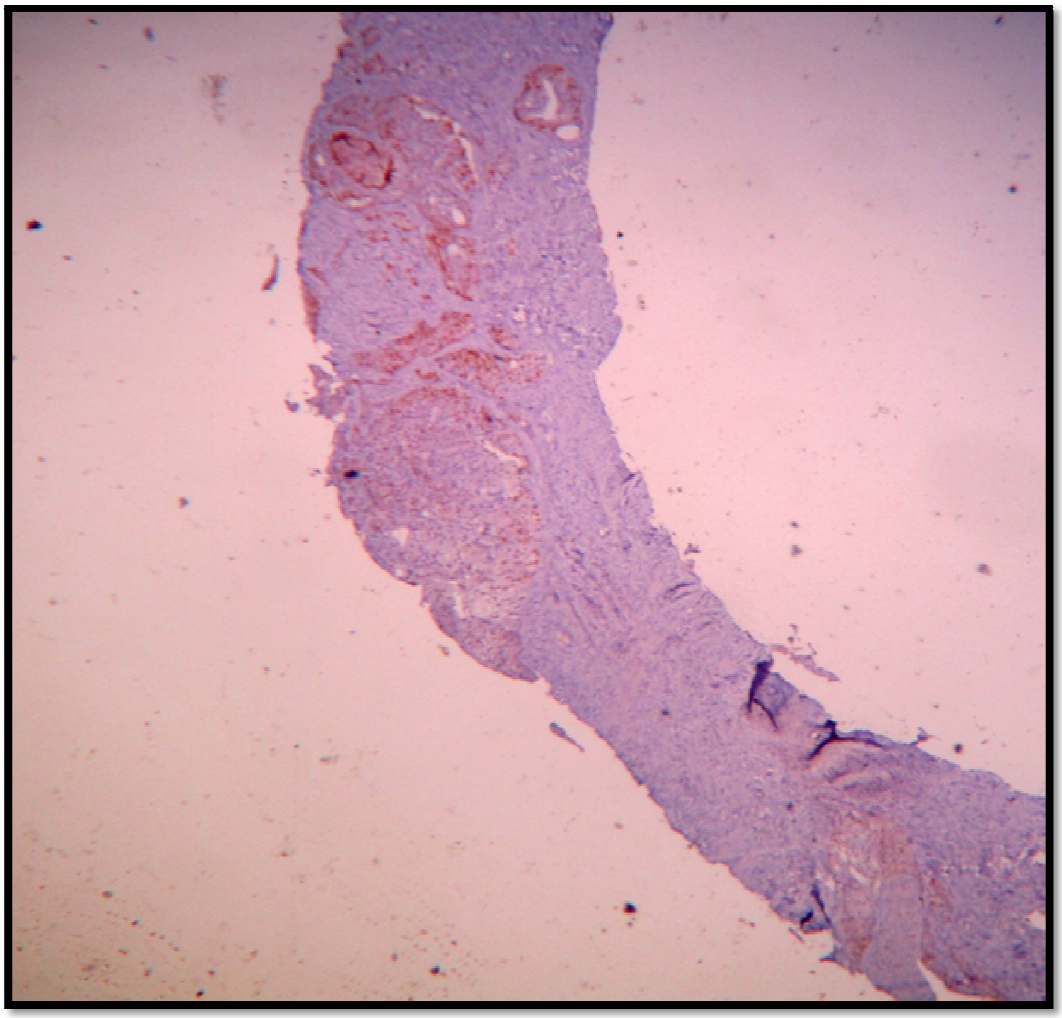


Fig.37- Nuclear expression of cyclin D1 in low grade prostate carcinoma (3+2=5) -10x.

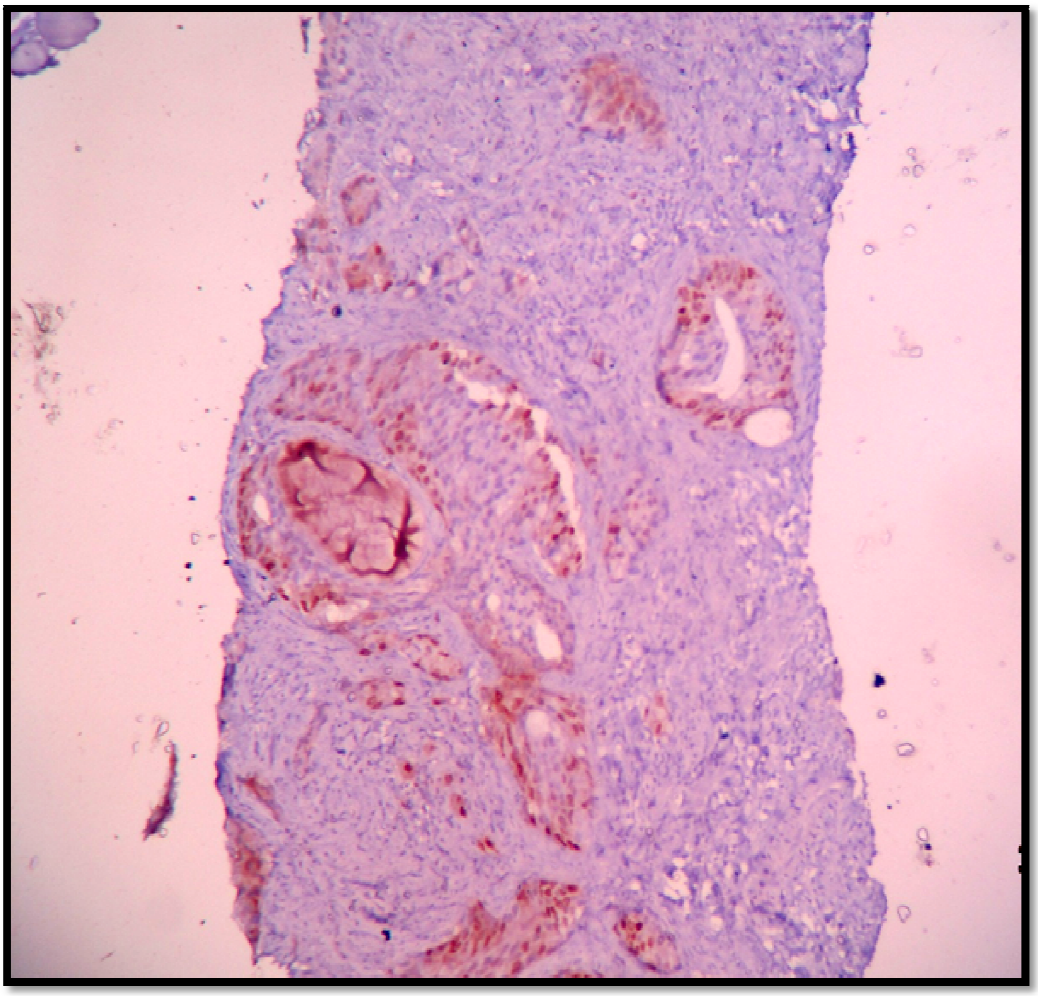


Fig .38 -Nuclear expression of cyclin D1 in low grade prostate carcinoma (3+2=5) -40x.

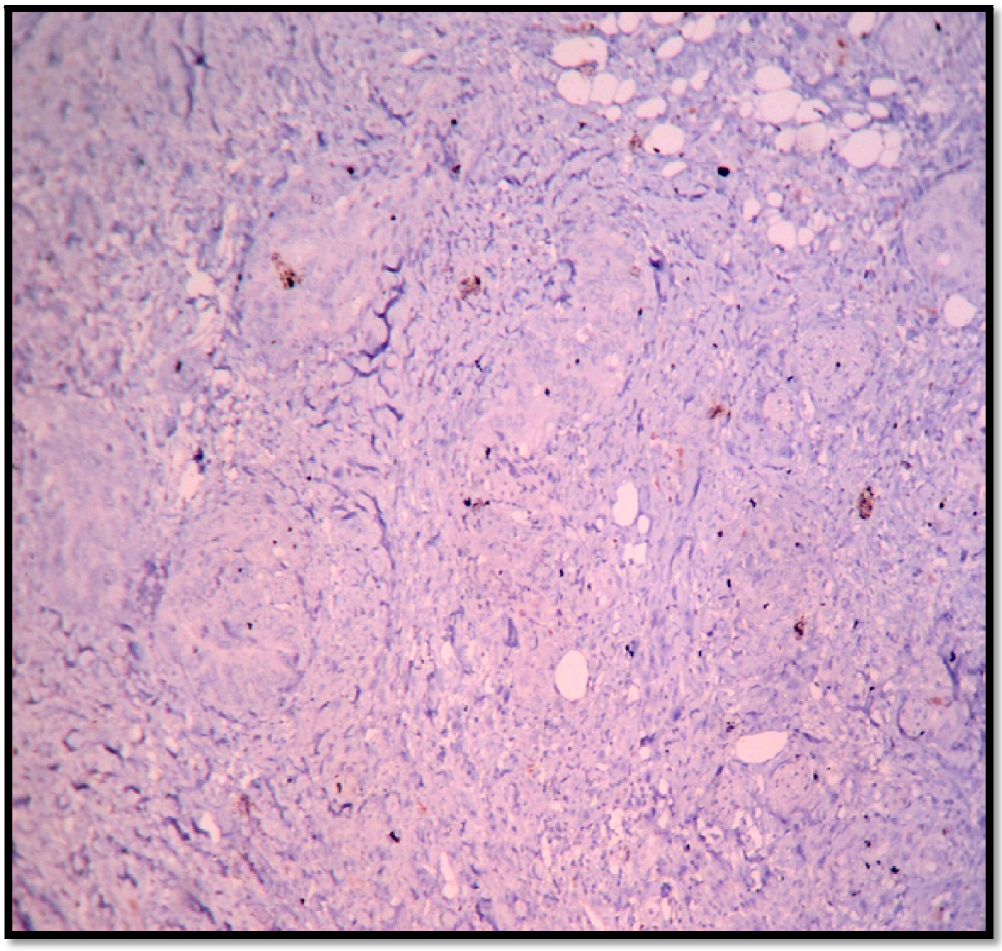


Fig.39 -Lack of nuclear expression of cyclin D1 in low grade prostate carcinoma (3+2=5) (10x)

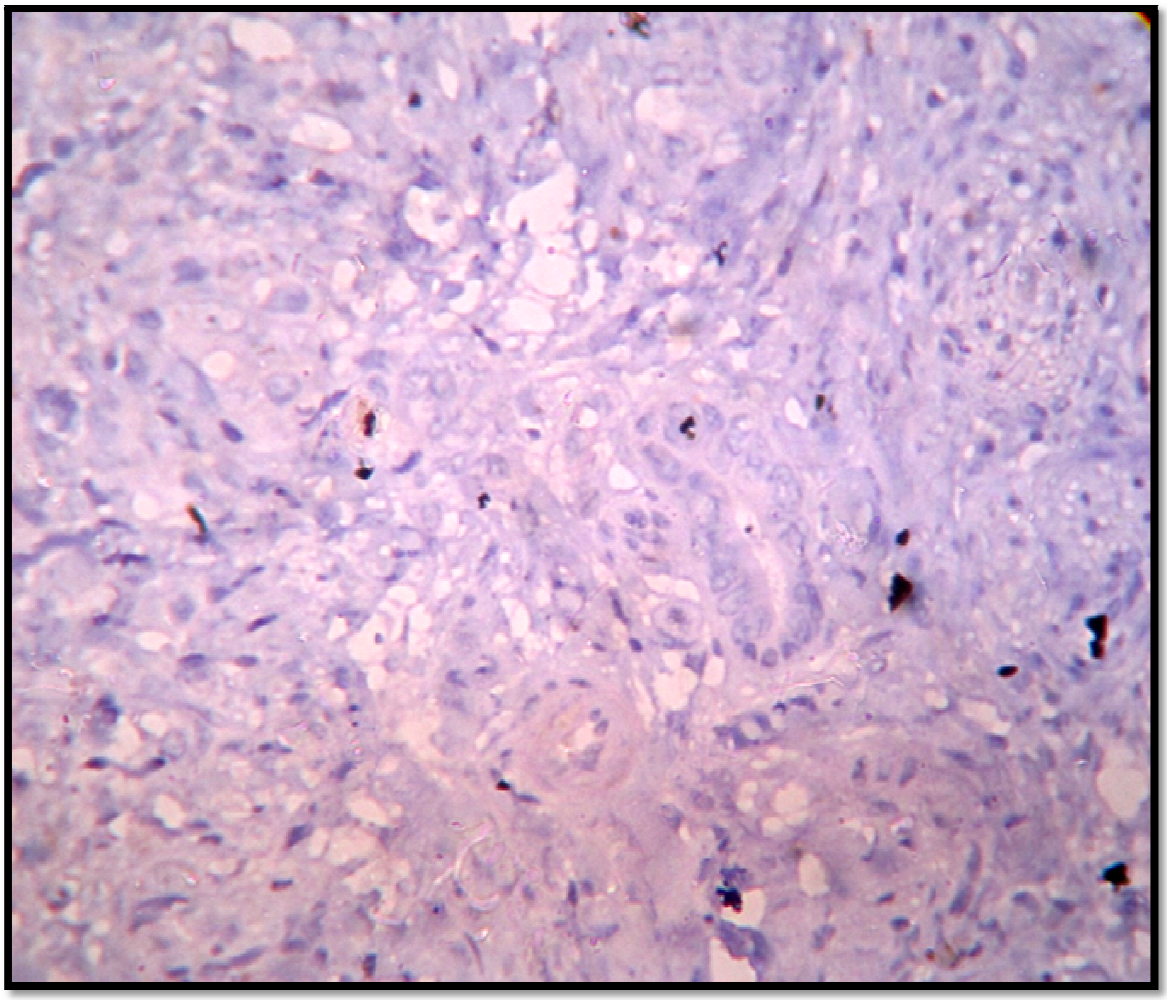


Fig.40 -Lack of nuclear expression of cyclin D1 in low grade prostate carcinoma (3+2=5) (40x)

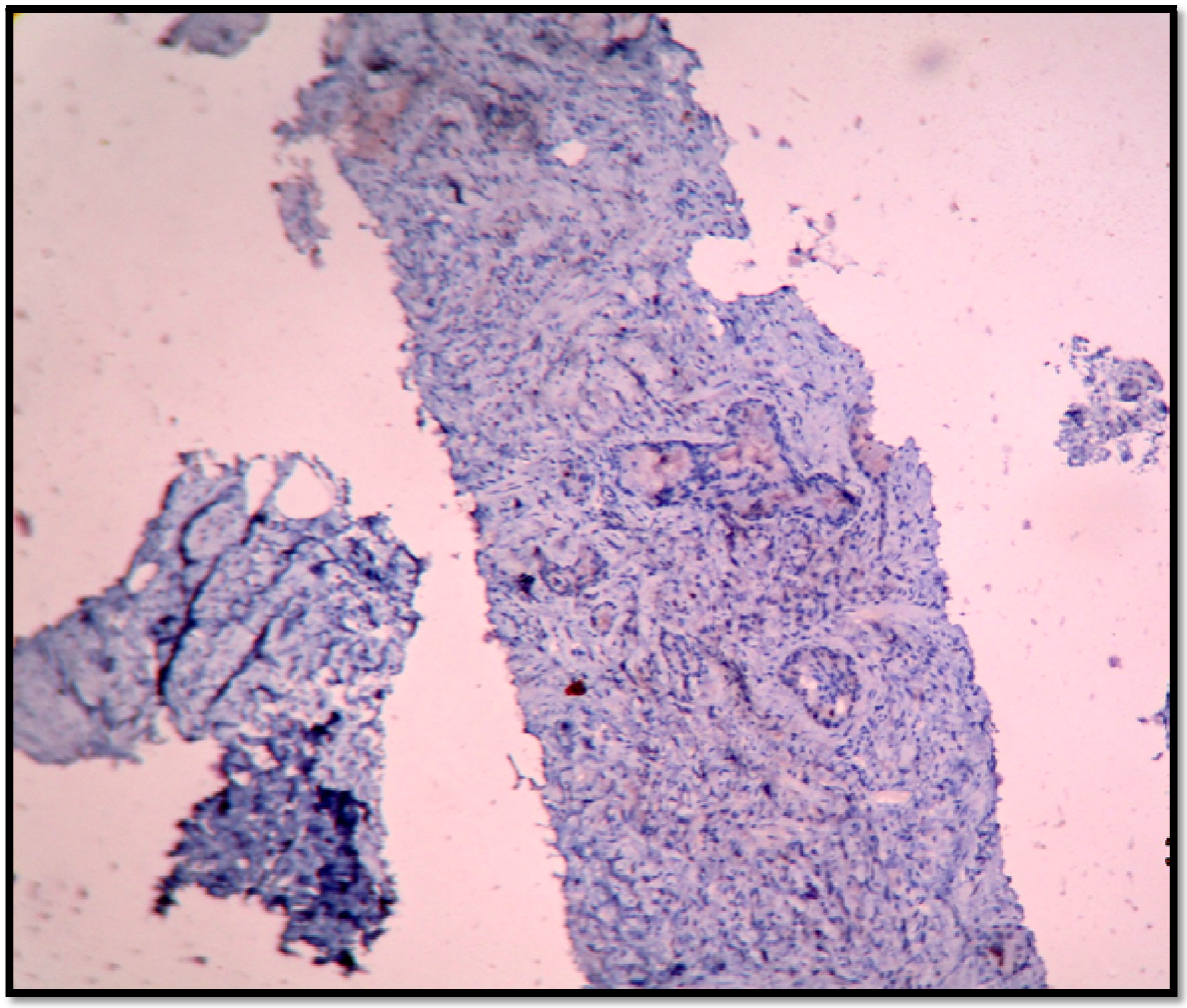


Fig.41- Lack of nuclear expression of cyclin D1 in low grade prostate adenocarcinoma (2+2=4) -10x

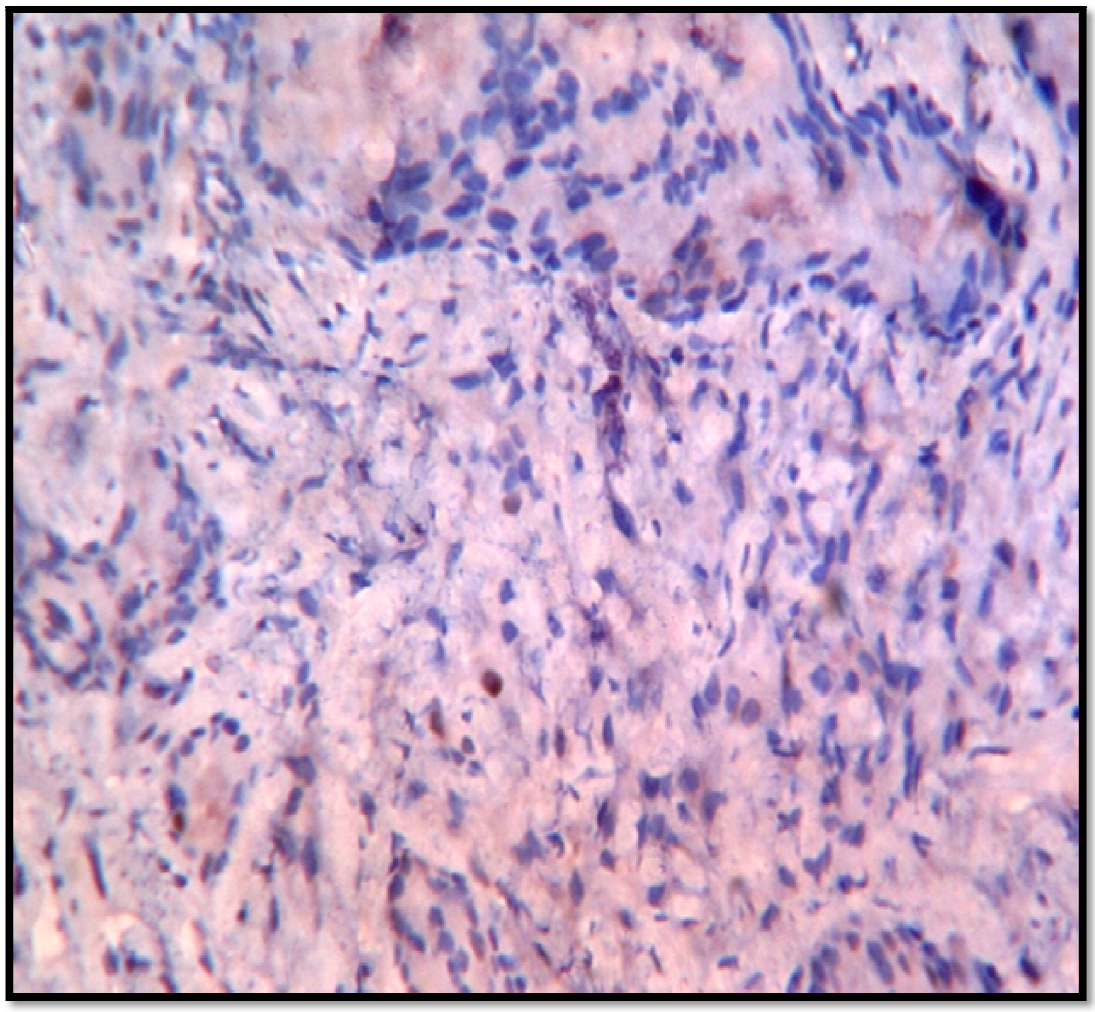


Fig.42 -Lack of nuclear expression of cyclin D1 in a low grade prostate adenocarcinoma (2+2=4)-40x

SIGNIFICANCE OF Ki67 EXPRESSION IN PROSTATE ADENOCARCINOMA

Ki67 is a proliferation associated nuclear antigen and is expressed in all cycling cells except for resting cells in the G0 phase .

Verma R. et al studied the significance of p53 and Ki-67 expression in prostate cancer. The study was conducted to find the immunohistochemical expression of p53 and Ki-67 as a prognostic factor in prostate carcinoma and **correlated their expression with Gleason's grade.**

A total of 50 prostate cancer cases were taken . Tumor grade was determined according to Gleason's grading system. Ki-67 expression was determined by IHC staining. The obtained results were analyzed using Spearman's statistical test. Carcinoma cases were graded histologically according to Gleason's grading system and Gleason's score was noted (2-4 - well differentiated , 5-7 -moderately differentiated ,8-10 - poorly differentiated tumors) . In this study older grading system was used , where Gleason scores 5 and 6 were included in moderately differentiated tumors , unlike the modified grading system where Gleason score 5 and 6 are included under well differentiated tumors .

The tumors were divided into five groups depending on the percentage of Ki-67 positive cells. Cases in which ≤ 2% of cells were stained were considered negative for Ki-67.Cases with Ki-67 index was ≤ 25% were considered 1+, 26-50% as 2+ 51-75% as 3+ and 76- 100% as 4+.

In this study all 4 (100%) well differentiated tumors were negative for Ki-67 expression. 19 of 31 (61.29%) moderately differentiated and 13 of 15 (86.66%) poorly differentiated tumors were positive for Ki-67 . **Thus, a statistically significant correlation was observed between Ki-67 positivity and increased Gleason's score in this study.**[32]

Madani SH et al conducted a study to find the frequency of Ki-67 and p53 expression among patients with prostate cancer. 49 cases were assessed . 3/49 cases (6.1%) were well differentiated ,21 (43%) moderately differentiated and the remaining 25 cases (51%) were poorly differentiated tumors. Ki-67 was negative in 14 case (28%).

All well differentiated tumors were negative for Ki-67. 8/ 21 (38%) cases of moderately differentiated and 3/25 (12%) of the poorly differentiated tumours were negative for Ki-67.Thus, 13/21 (62%) cases of moderately differentiated and 21/25 (84%) cases of poorly differentiated prostate cancers showed Ki-67 positivity.

Hence, a statistically significant relation was found between the increased Ki-67 labeling index and increased Gleason's grade .[33]

In the present study which includes 50 cases , IHC was done using Ki-67 stain. 25/ 50 cases (50%) stained positively with Ki-67. Here tumors with scores 5 and 6 were grouped under well differentiated tumors. Thus , tumors with low grade included tumors with Gleason's score ≤ 6 and those with a score ≥ 7 were grouped as high grade tumors .

A significant correlation was found between Ki-67 positivity and high Gleason's score in this study also.

15/16 (94% cases) of high grade tumors (Gleason's score ≥ 7) showed Ki-67 positivity in the present study. 10/ 34 (only 29%) of low grade cancers showed Ki67 positivity.

COMPARISON OF Ki-67 EXPRESSION IN PRESENT STUDY WITH STUDIES CONDUCTED BY VERMA ET AL AND MADANI SH ET AL

Similar to the studies conducted by Verma et al and Madani SH et al, 10/10 well differentiated prostate adenocarcinoma (Gleason's score 2-4) cases (100%) showed no Ki-67 expression. 19/34 (39%) moderately differentiated tumors with Gleason's score between 5-7 and all i.e. 6/6 (100%) poorly differentiated adenocarcinomas showed Ki-67 positivity.

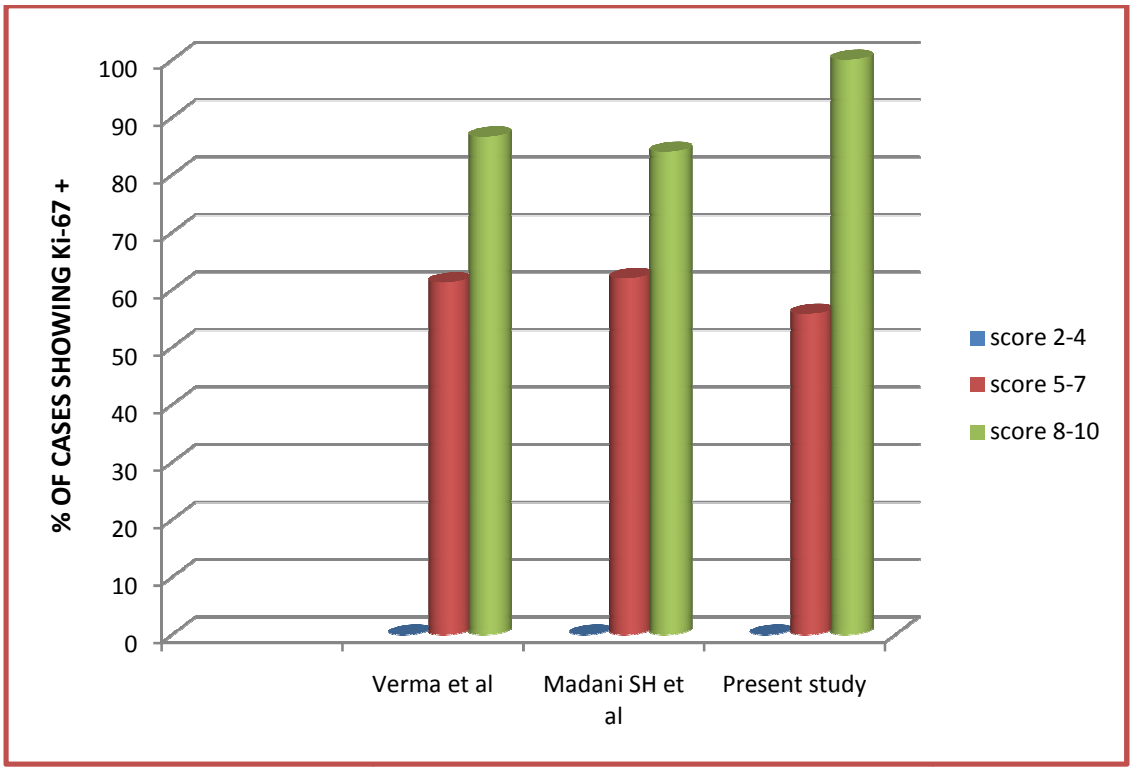
Number of cases with well differentiated , moderately differentiated and poorly differentiated adenocarcicomas showing Ki-67 expression is depicted in figure 43. Figures 44-49 show high grade tumors expressing Ki-67 .Figures 50-55 show low grade prostate adenocarcinomas lacking Ki-67 expression.

S.NO.	AUTHOR	NO. OF CASES WITH GLEASON SCORE 2-4	NO . OF CASES WITH SCORE 2-4 SHOWING Ki -67+	% OF CASES SHOWING Ki-67 +
1.	Verma R et al	4	0	0
2.	Madani SH et al	3	0	0
3.	Present study	10	0	0

S.NO.	AUTHOR	NO. OF CASES WITH GLEASON SCORE 5-7	NO . OF CASES WITH SCORE 5-7 SHOWING Ki -67+	% OF CASES SHOWING Ki-67 +
1.	Verma R et al	31	19	61.2
2.	Madani SH et al	21	13	62
3.	Present study	34	19	55.8

S.NO.	AUTHOR	NO. OF CASES WITH GLEASON SCORE 8-10	NO . OF CASES WITH SCORE 8- 10 SHOWING Ki -67+	% OF CASES SHOWING Ki-67 +
1.	Verma R et al	15	13	86.66
2.	Madani SH et al	25	21	84
3.	Present study	6	6	100

Fig .43-COMPARISON OF Ki67 POSITIVITY BETWEEN DIFFERENT GRADES OF PROSTATE ADENOCARCINOMAS.



COMPARISON OF Ki-67 POSITIVITY BETWEEN LOW GRADE AND HIGH GRADE PROSTATE ADENOCARCINOMA CASES

PRESENT STUDY USING MODIFIED GLEASON SCORING SYSTEM	NO . OF LOW GRADE CASES (≤ 7) SHOWING Ki-67 +	NO. OF HIGH GRADE CASES (≥7) SHOWING Ki-67 +
50	10/34 (29%)	15/16 (94%)

KI-67 EXPRESSION	HIGH GRADE CASES	LOW GRADE CASES	TOTAL CASES	P -value
Ki-67 + CASES	15	10	25	0.0002<0.001 (p < 0.001)
Ki-67 - CASES	1	24	25	
Total	16	34	50	

Thus, in the present study a statistically significant correlation is found between Ki -67 nuclear positivity and increasing Gleason's score.

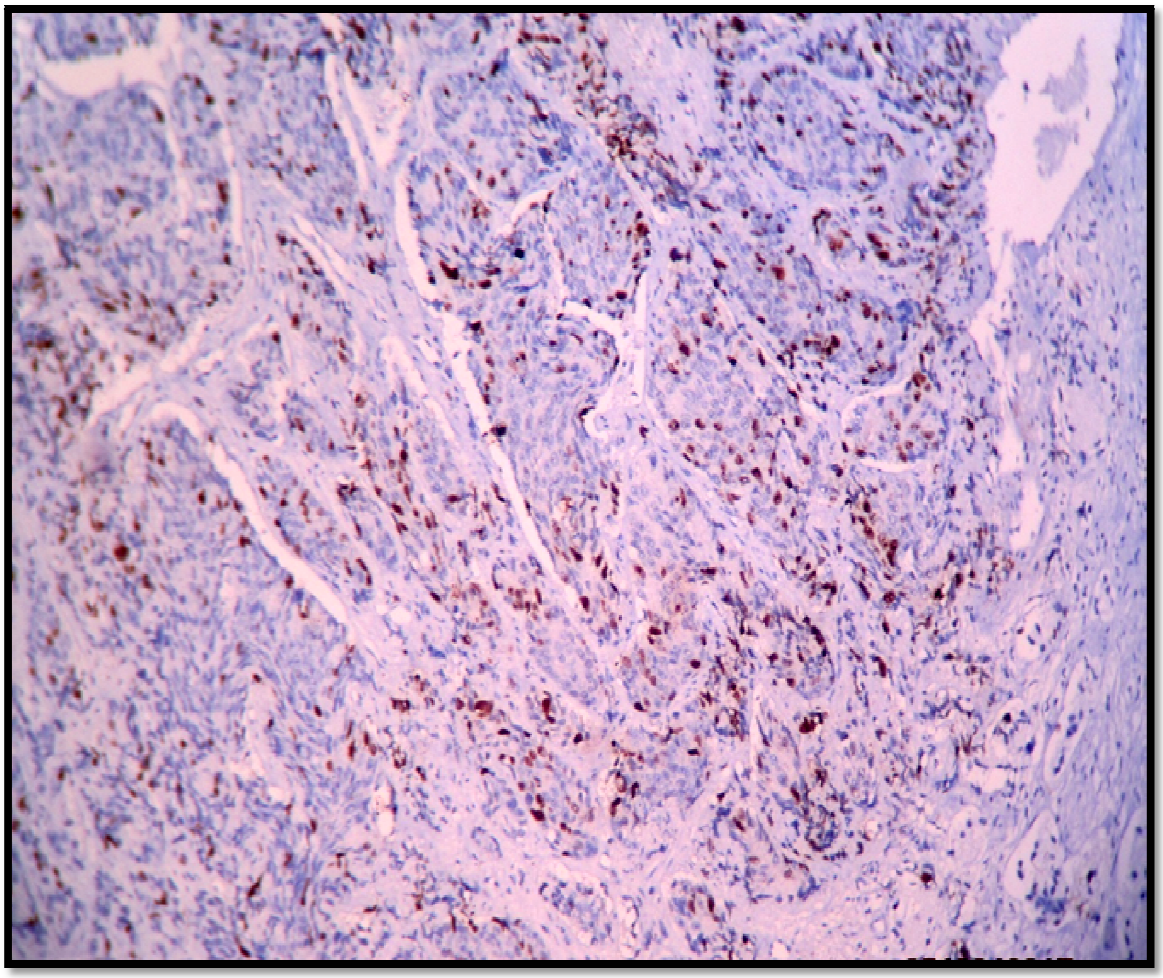


Fig.44-Nuclear expression of Ki-67 in high grade prostate carcinoma (4+5=9) - 10x.

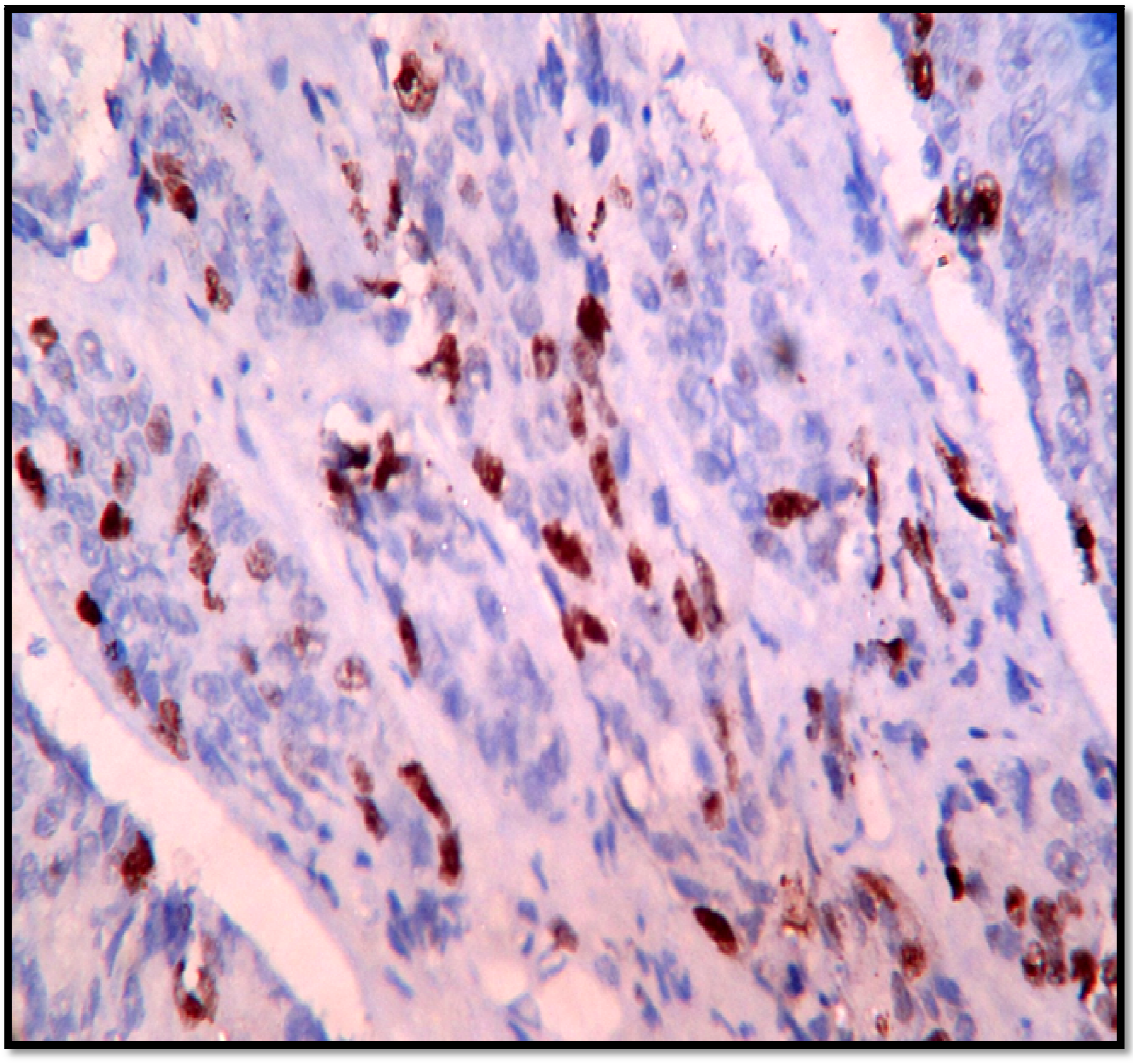


Fig. 45 -Nuclear expression of Ki-67 in high grade prostate carcinoma (4+5=9) - 40x

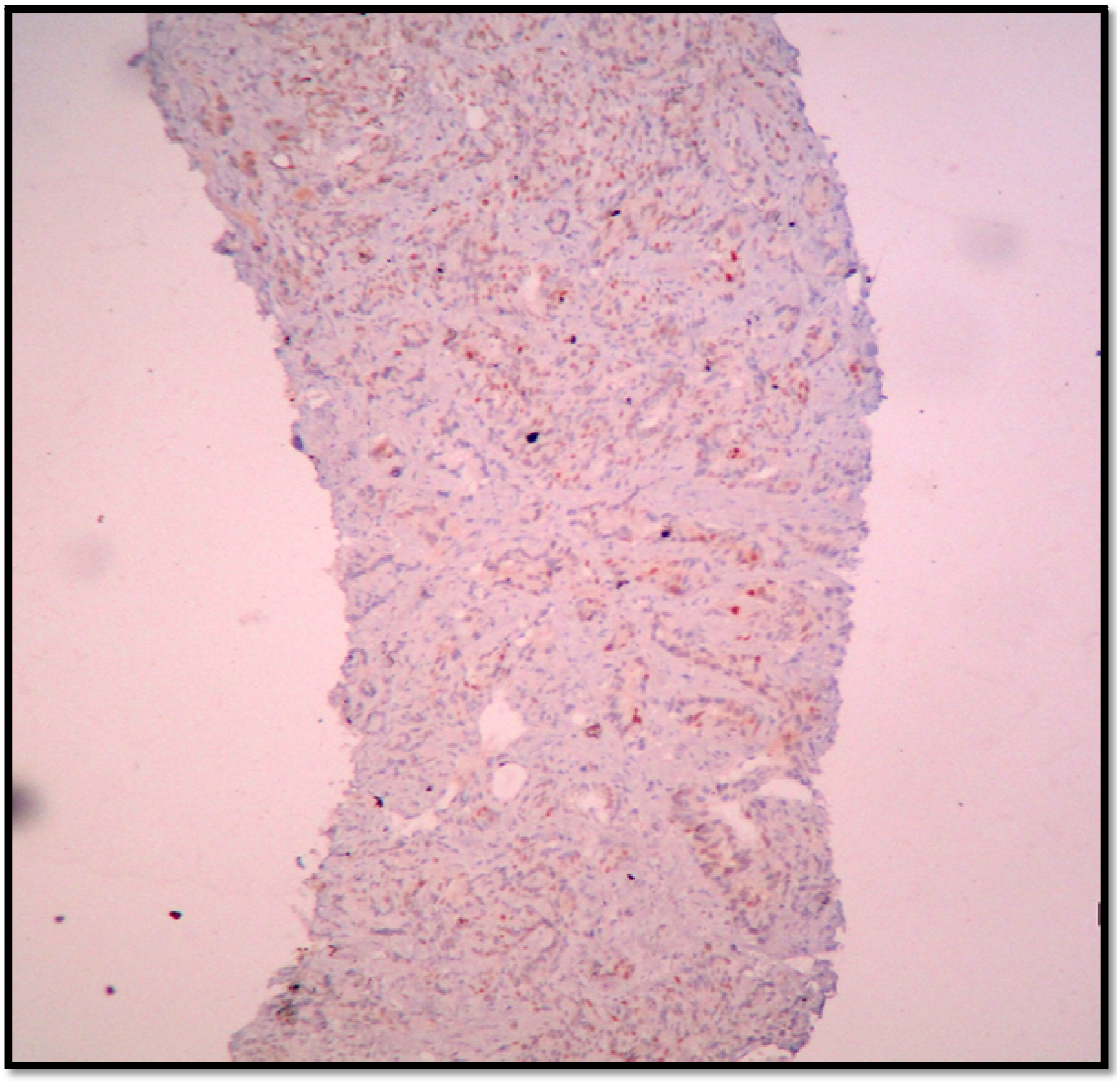


Fig .46- Nuclear expression of Ki-67 in high grade prostate carcinoma (5+4=9) - 10x

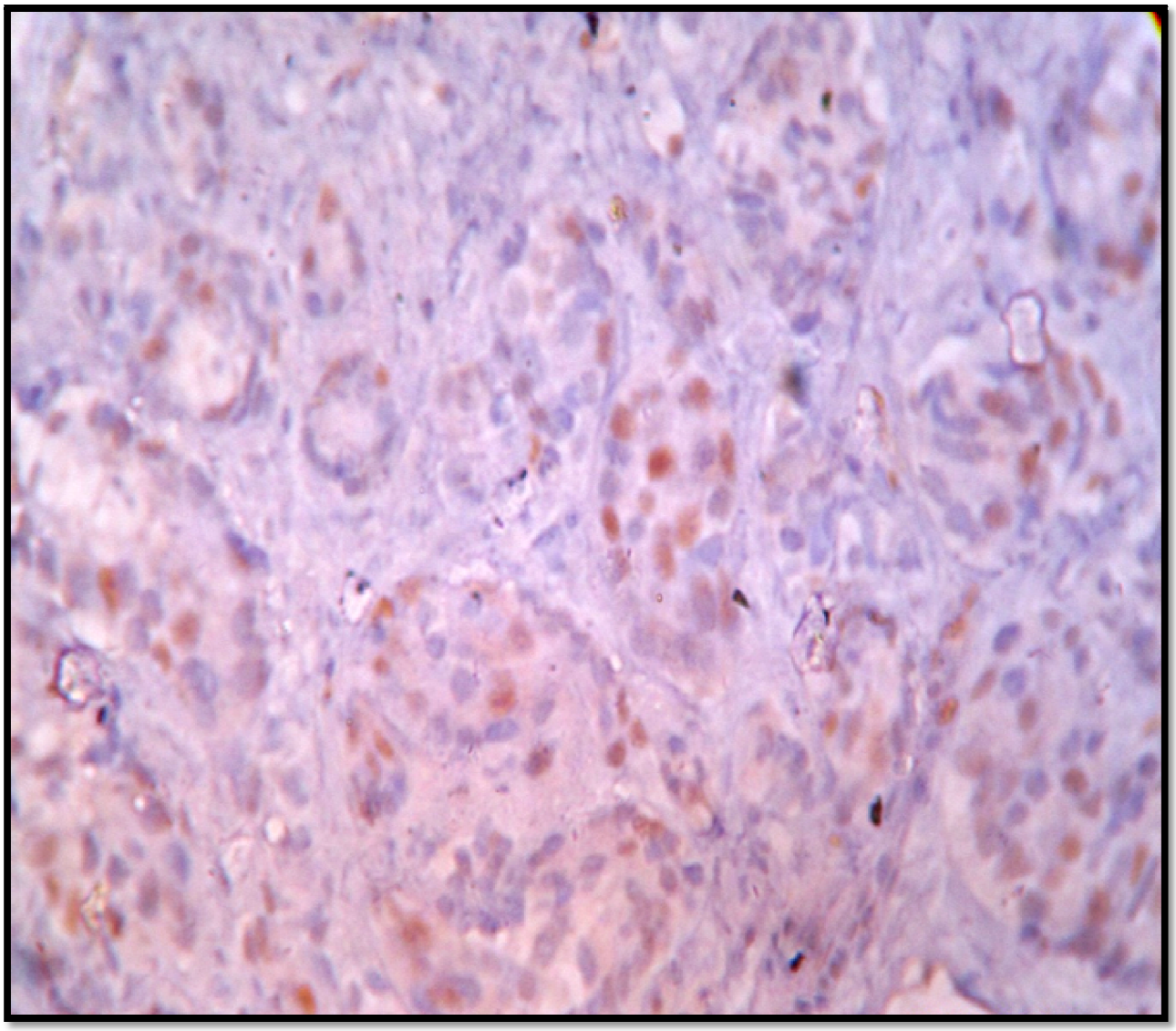


Fig .47- Nuclear expression of Ki-67 in high grade prostate carcinoma (5+4=9) - 40x

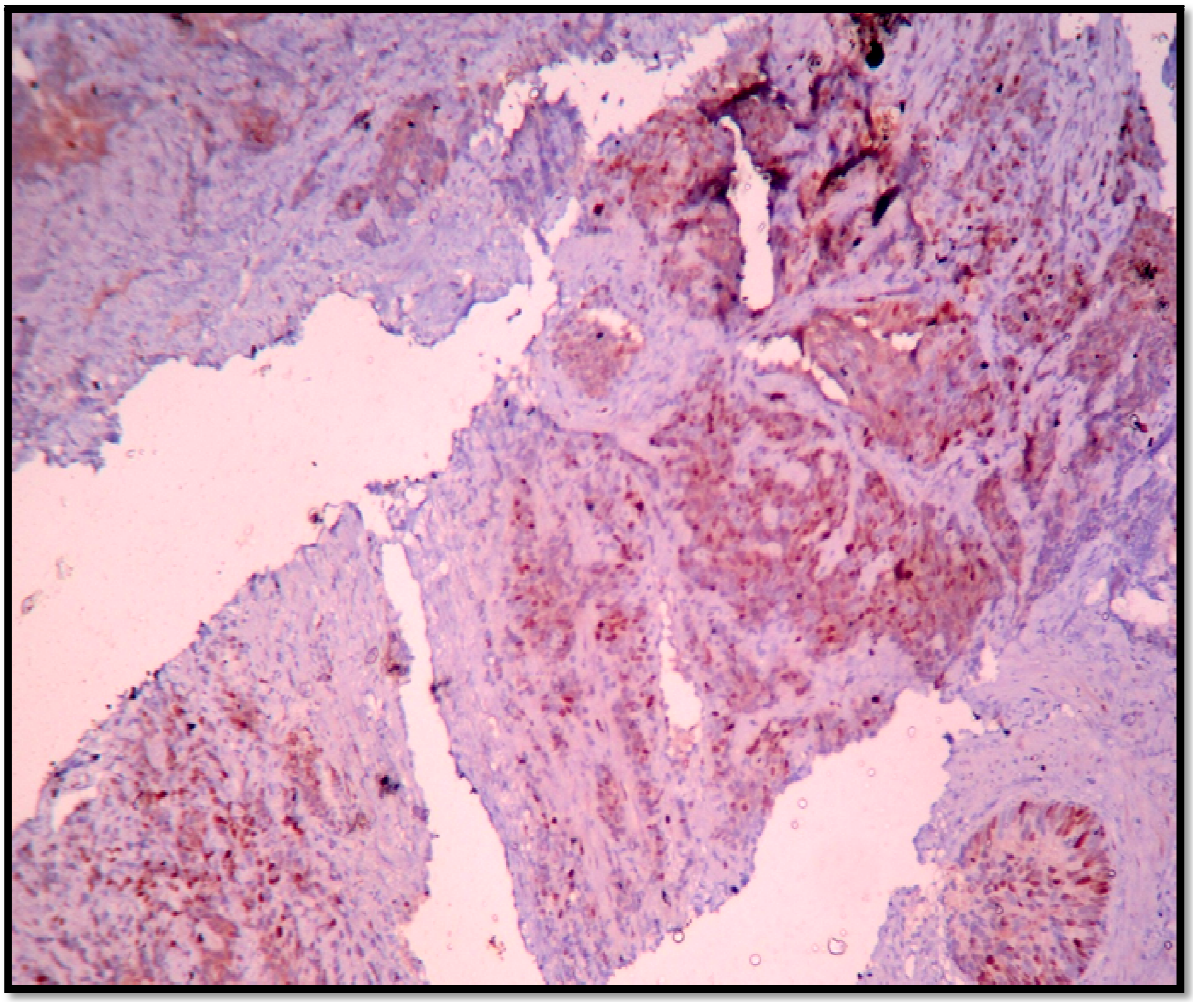


Fig .48 Nuclear expression of Ki-67 in high grade prostate carcinoma (3+4=7) - 10x

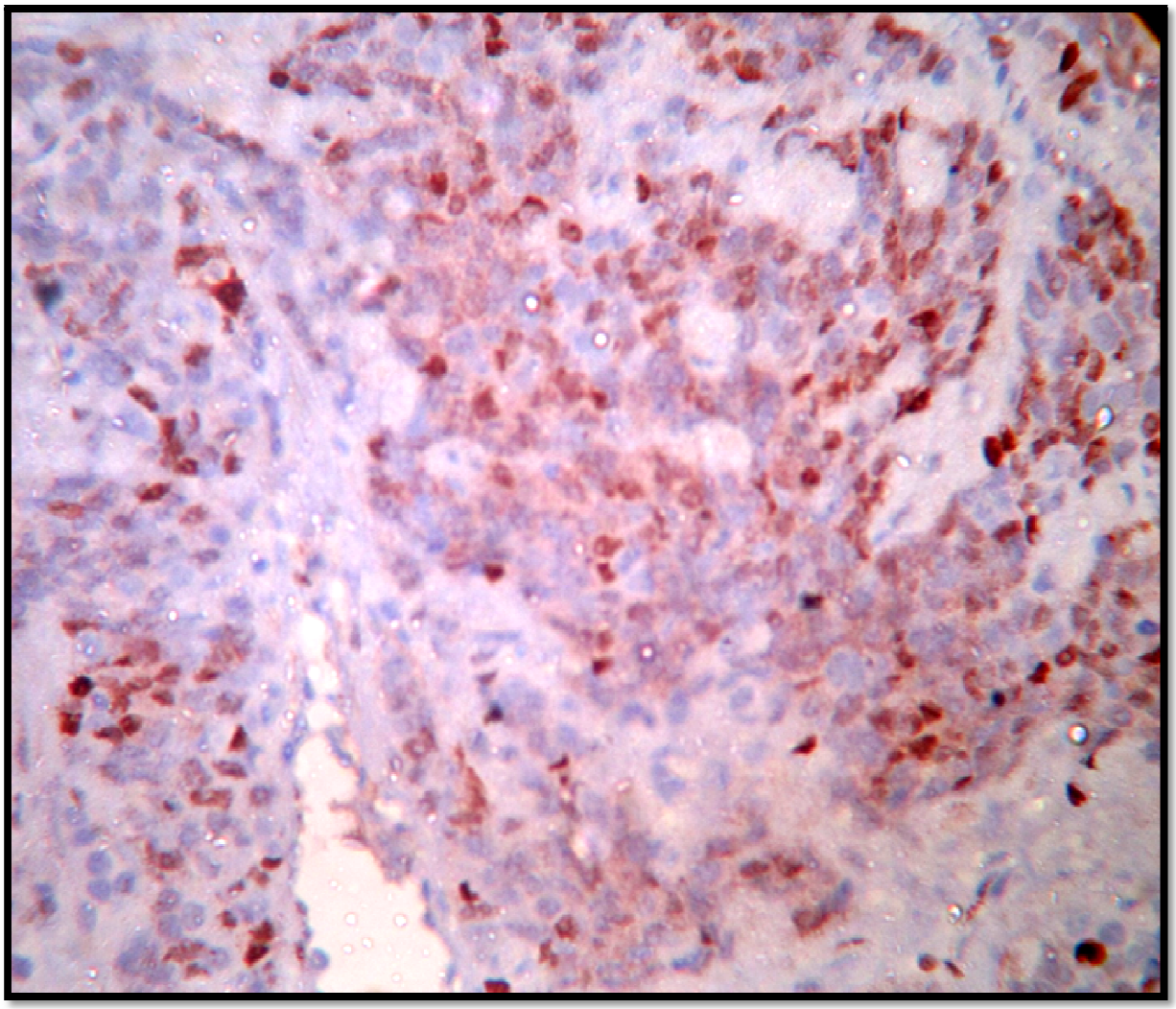


Fig.49 - Nuclear expression of Ki-67 in high grade prostate carcinoma (3+4=7) - 40x

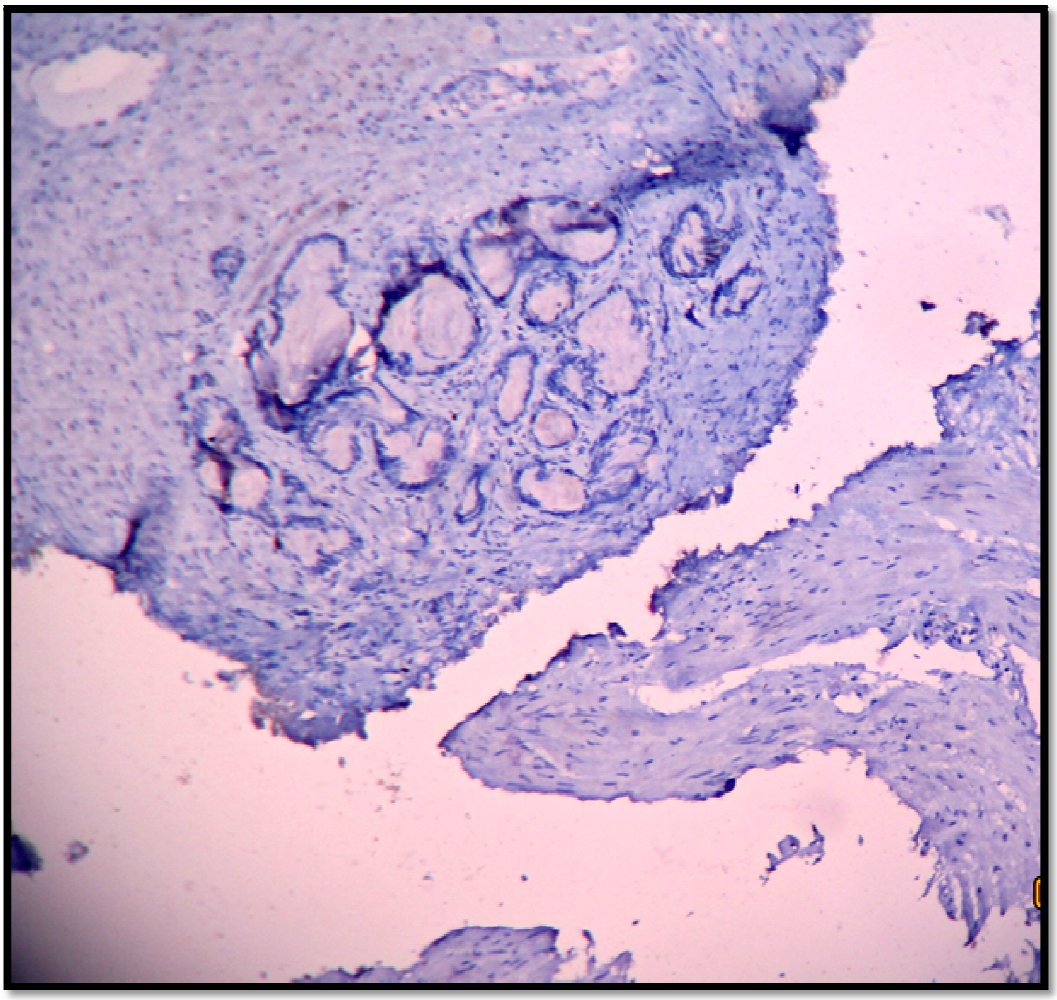


Fig .50-Lack of nuclear expression of Ki-67 in low grade prostate carcinoma (score=2+1=3)- 10x

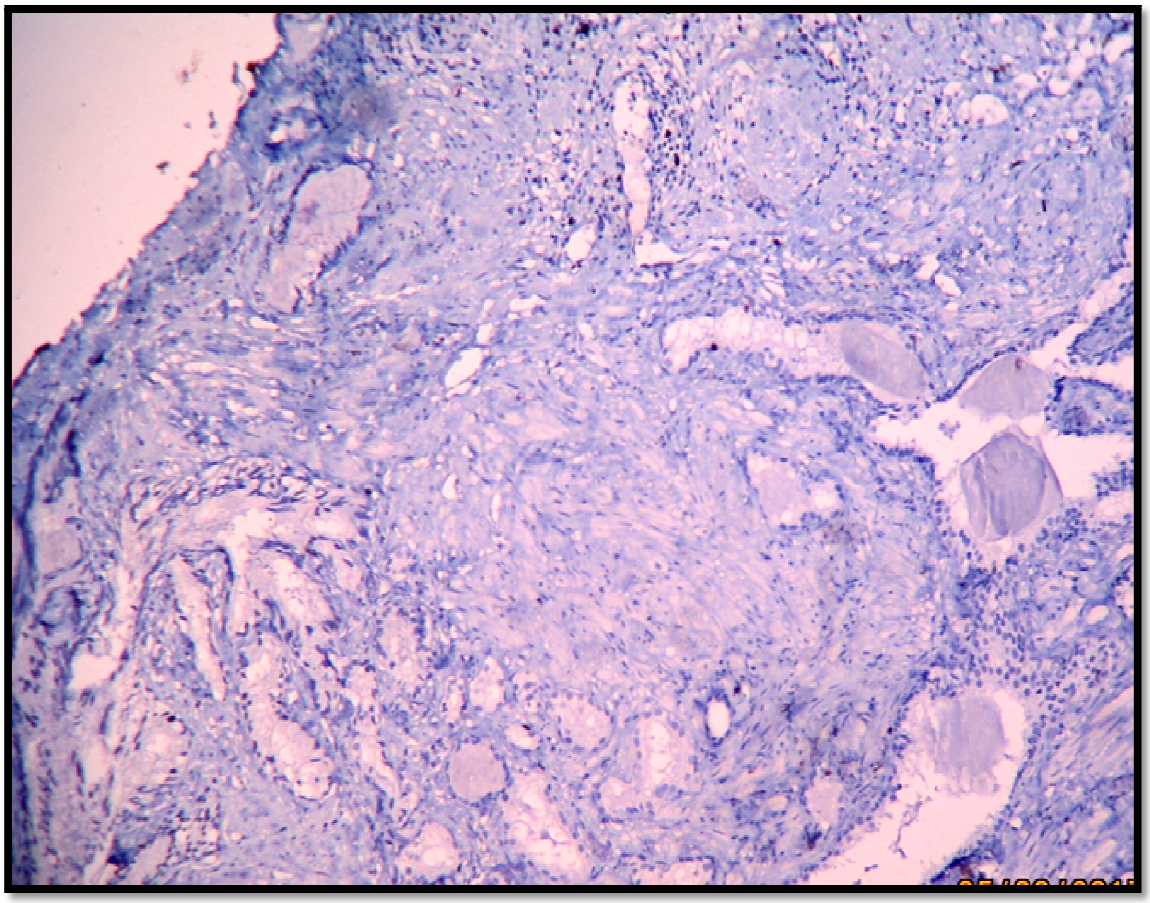


Fig .51 -Lack of nuclear expression of Ki-67 in low grade prostate adenocarcinoma -score 2+1=3 (40x)

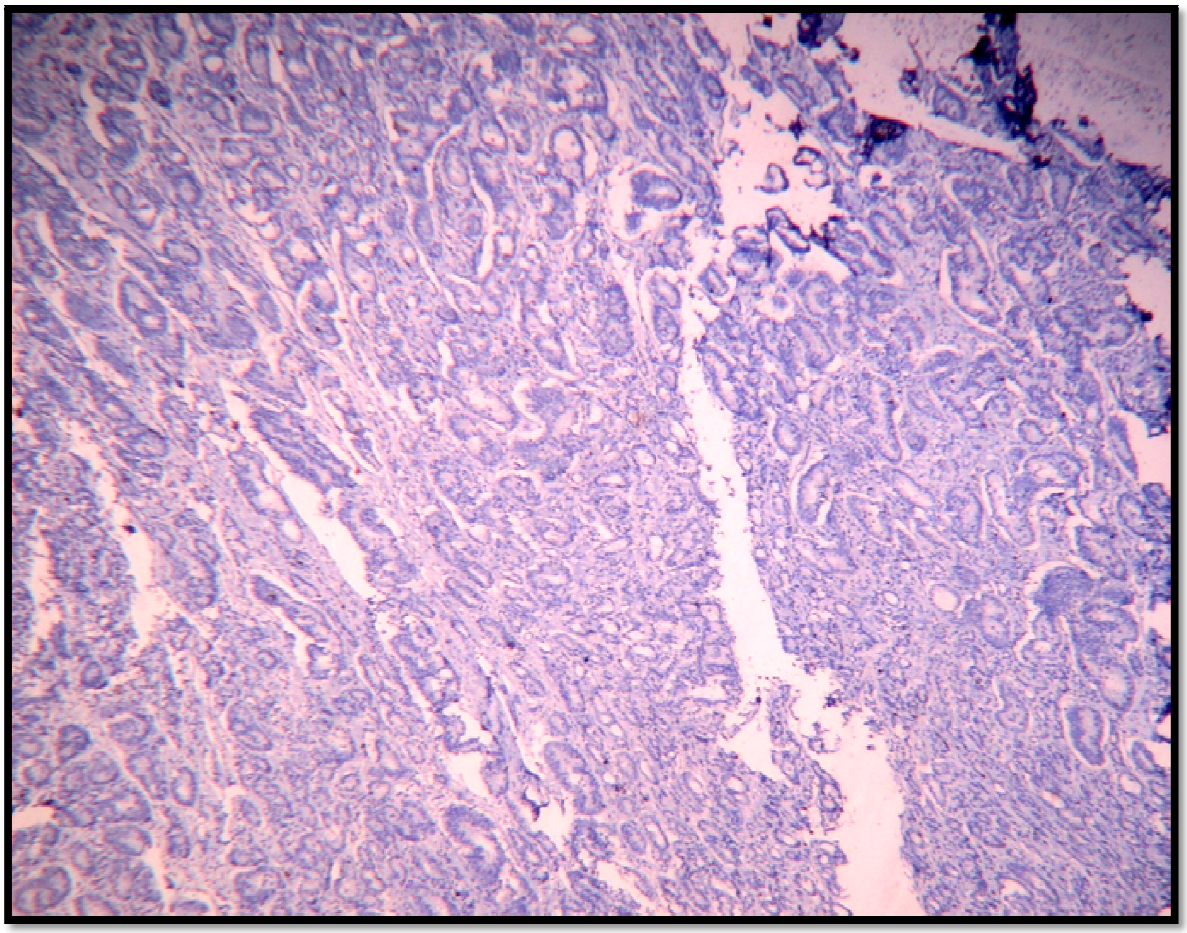


Fig.52 -Lack of nuclear expression of Ki-67 in low grade prostate carcinoma (score=3+3=6)- 10x

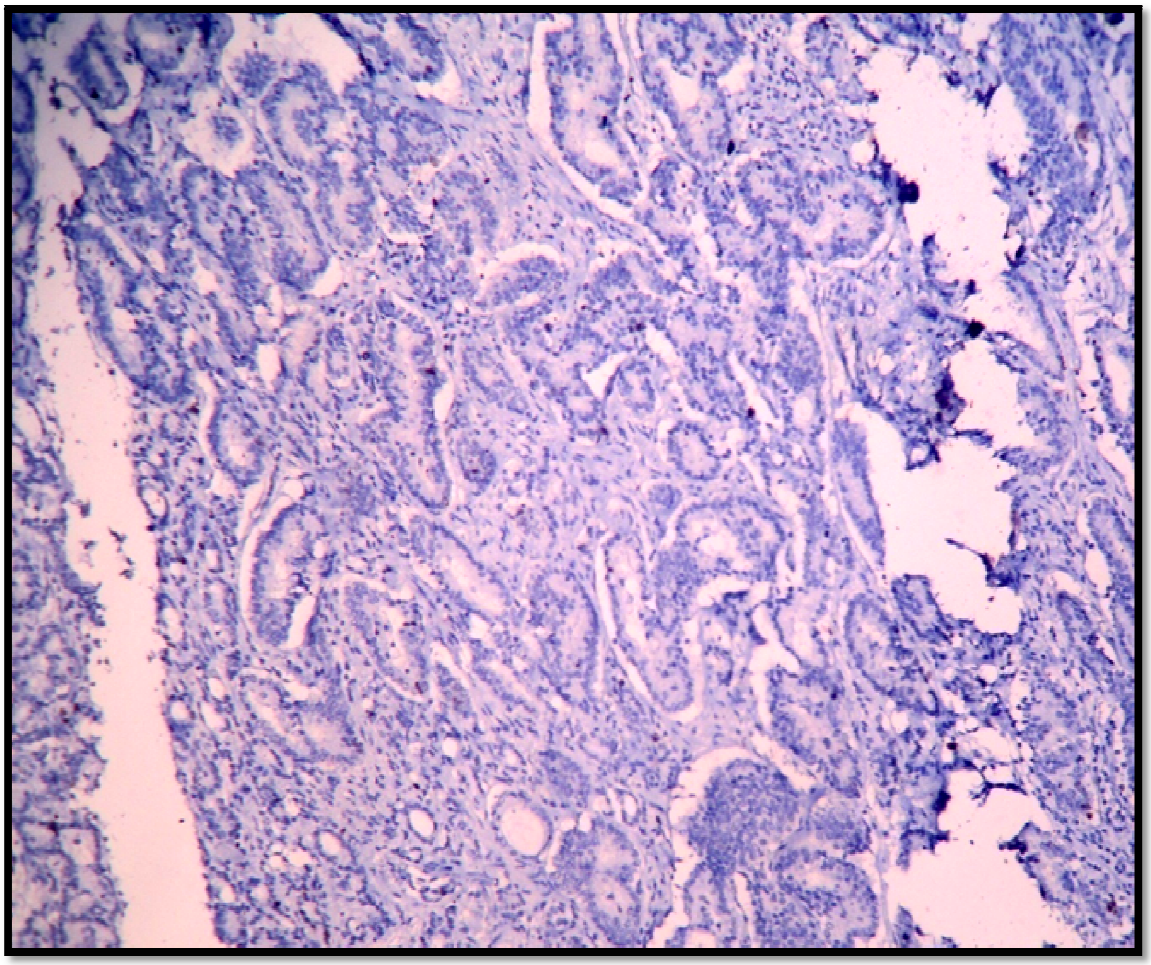


Fig.53--Lack of nuclear expression of Ki-67 in low grade prostate carcinoma (score=3+3=6)-40x

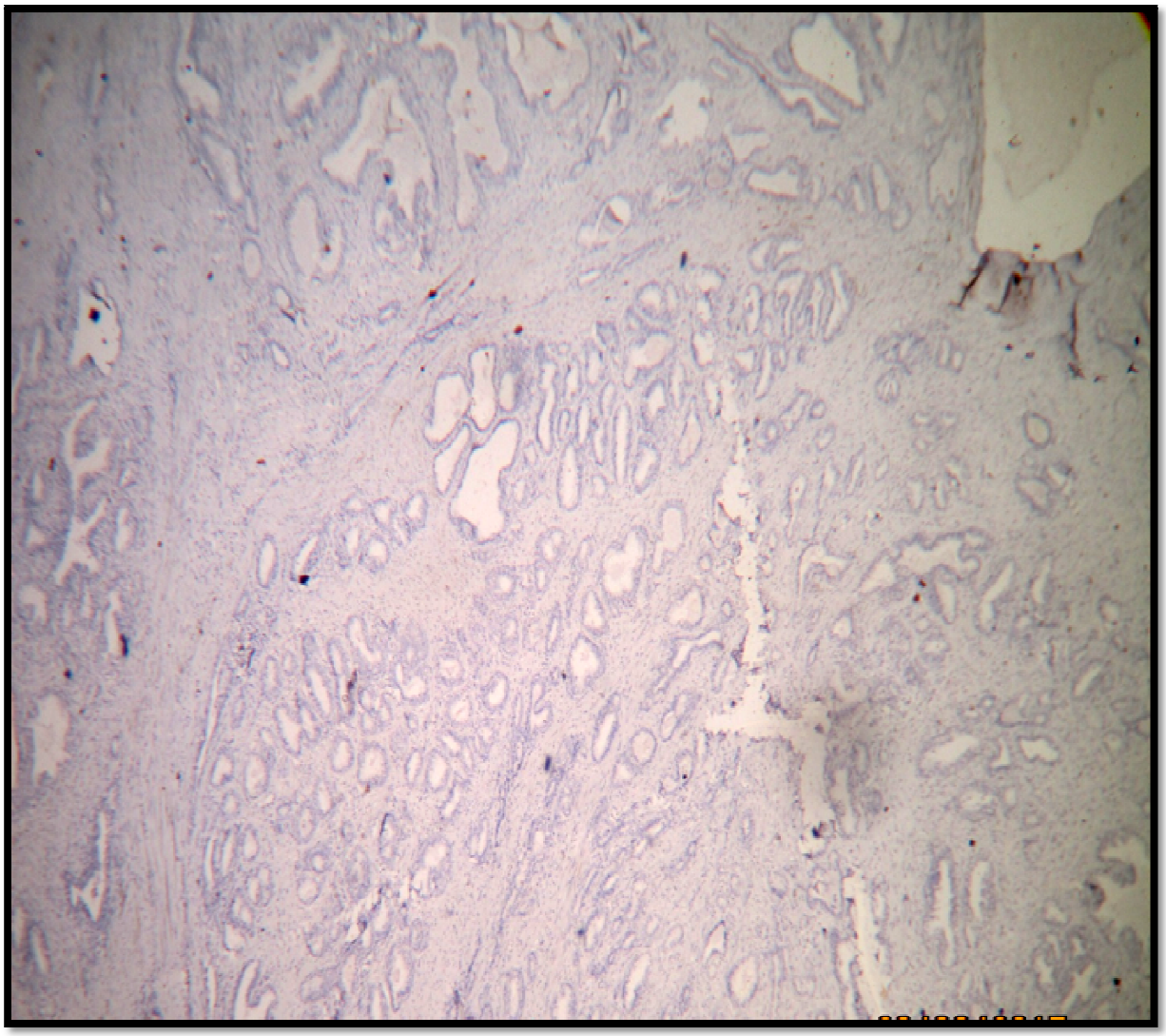


Fig .54- Lack of nuclear expression of Ki-67 in a low grade prostate adenocarcinoma (3+2=5)- 10x

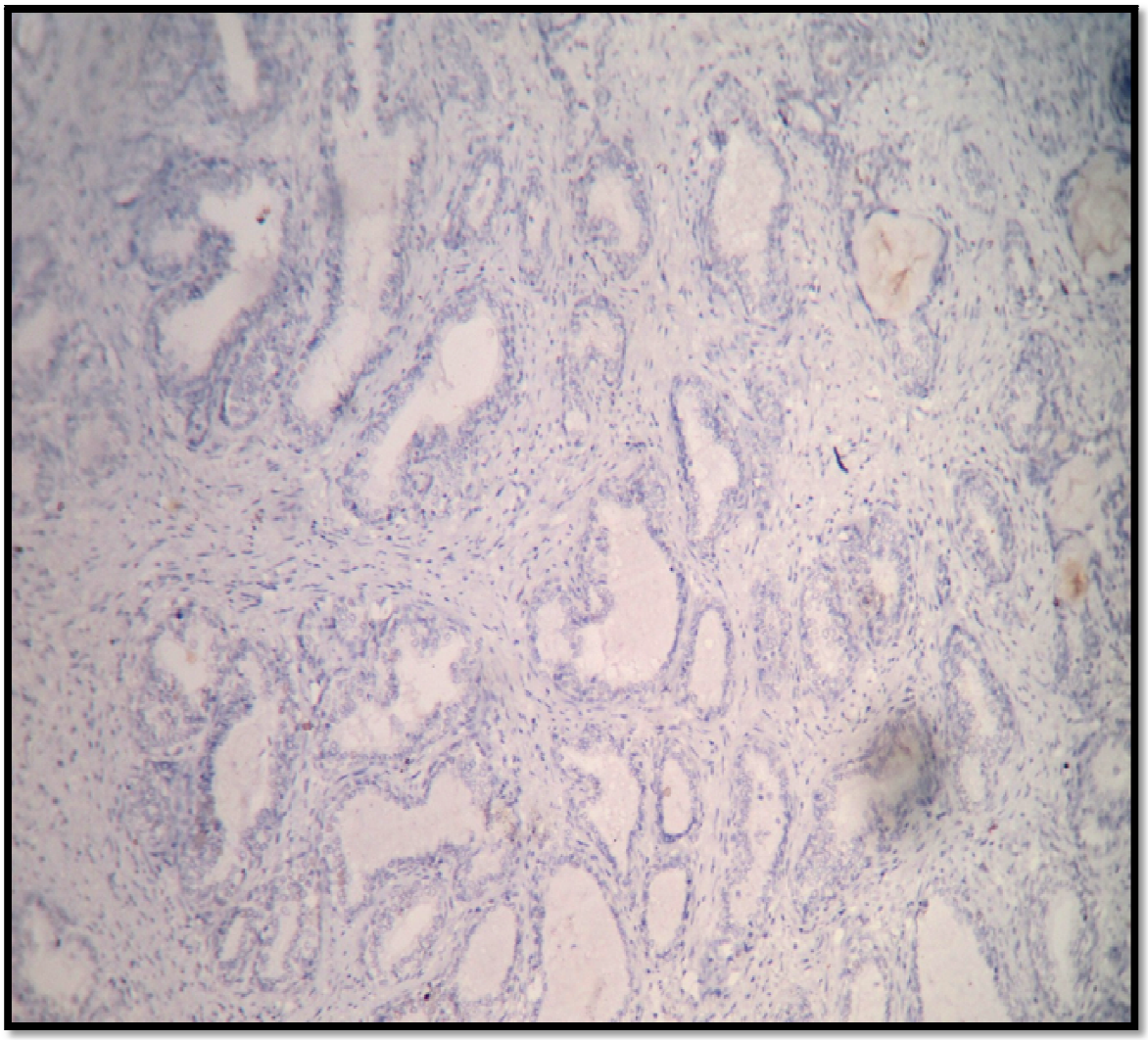


Fig.55-Lack of nuclear expression of Ki-67 in a low grade prostate adenocarcinoma (3+2=5)- 40x

ER-β EXPRESSION IN PROSTATE ADENOCARCINOMA

Asgari M . et al stated that estrogen induction of cell proliferation is a crucial step in carcinogenesis of gynecologic target tissues . Many studies have been have been recently done, showing that prostate cancer growth is influenced by estrogen. A study was conducted by them to investigate the ER-β expression in human prostate cancer tissues.52 paraffin -embedded blocks of prostate needle biopsies were selected Frequency and rate of ER-β expression in different grades of prostate adenocarcinoma according to Gleason grading system was determined. IHC of tissue sections was performed using monoclonal anti ER-b antibody.

The tumors were categorized as " low grade " if Gleason score was ≤ 4. Gleason score of 5, 6 and 7 was considered as " intermediate grade" and "high grade" if the Gleason score was ≥ 8. ER- b expression was assigned as positive when >10% of tumoral nuclei were stained. Rate of ER-b expression was defined as percentage of positive nuclei per 200 cells count. Chi square test was used for comparative analysis.

ER-b expression was noticed in the tumoral cells of prostate carcinoma in all 29 cases with low and intermediate tumors (100%) and 19 of 23 cases with high grade tumor (83%). Mean rate of expression of ER- b expression in low and intermediate grade cancers was 64.81% whereas high grade of cancers showed 49.48% rate of expression. Thus, ER- b expression was reduced in high grade prostate cancers compared to low and intermediate grade ones. [35]

In the present study with 50 cases, IHC was done using ER-b antibody. Grouping the cases into low (score 2-4), intermediate (score 5-7) and high grade (8-10) as in the study conducted by Asgari M. et al, the number of low and intermediate grade cases showing ER-b expression were compared with the number of high grade cases showing ER-b expression .

Also the mean expression of ER-b among low and intermediate grade tumors was compared with that of high grade tumors.

It was found that 34/44 (77%) low and intermediate grade tumors (score 2-4 & 5-7) showed ER -b expression . None of the high grade tumors showed ER-b expression .Also mean expression of ERb in low , intermediate grade tumors was 26.13% and was calculated by summing up the expression of ER-b in all 44 low and intermediate grade cases and dividing it by total number of cases with low & intermediate grade tumors none of the high grade tumors showed ERb expression.

S.NO	AUTHORS	NO. OF LOW AND INTERMEDIATE GRADE TUMORS SHOWING ER-β +	NO. OF HIGH GRADE TUMORS SHOWING ERβ+	MEAN EXPRESSION OF ER-β IN LOW & INTERMEDIATE GRADE TUMORS	MEAN EXPRESSION OF ER-β IN HIGH GRADE TUMORS
1.	Asgari M.et al	29/29 -100%	19/23- 83%	64.81%	49.48%
2.	Present study	34/44- 77%	0/6 -0%	26.13%	0%

This table shows that as the grade of the tumor increases , nuclear reactivity for ER-b decreases which correlates with study conducted by Asgari M . et al. In the present study none of the high grade tumors show ER-beta expression.

COMPARISON OF ER b EXPRESSION BETWEEN LOW & HIGH GRADE TUMORS USING MODIFIED GLEASON SCORING SYSTEM

NO.OF CASES IN PRESENT STUDY	NO. OF LOW GRADE (2-6) CASES SHOWING ER b +	NO. OF HIGH GRADE (7-10) CASES SHOWING ER b +	MEAN EXPRESSION OF ER b IN LOW GRADE TUMORS	MEAN EXPRESSION OF ER b IN HIGH GRADE TUMORS
50	**29/34- 85.29%**	**5/16 - 31.25%**	**30.58%**	**7%**

The above table shows the difference in proportion of low grade (Gleason score ≤6) and high grade tumors (Gleason score ≥ 7) showing ER- b expression using modified Gleason's grading system which does not include scores 5 and 6 in intermediate group , instead prostate adenocarcinomas with Gleason's score 5 and 6 are included under the group of well differentiated (low grade) tumors. And also difference in the mean expression of ER b between the low and high grade tumors is depicted in the table below.

ERb EXPRESSION	LOW GRADE CASES (2-6)	HIGH GRADE CASES (7-10)	TOTAL CASES	P-VALUE
ER b + CASES	29	5	34	0.000132<0.001 (p< 0.001)
ER b - CASES	5	11	16	
Total	34	16	50	

Thus, a statistically significant (p value < 0.001) correlation is found between ER -b expression and nuclear expression . Nuclear expression of ER - b decreases with increase in the grade of the tumor.

Figure 56 shows a comparison in ER- b expression between low / intermediate and high grade tumors.

Figure 57 depicts the mean expression of ER-b between low and high grade prostate adenocarcinomas.

Fig. 56 -COMPARISON OF MEAN EXPRESSION OF ER-b BETWEEN LOW/ INTERMEDIATE GRADE AND HIGH GRADE TUMORS.

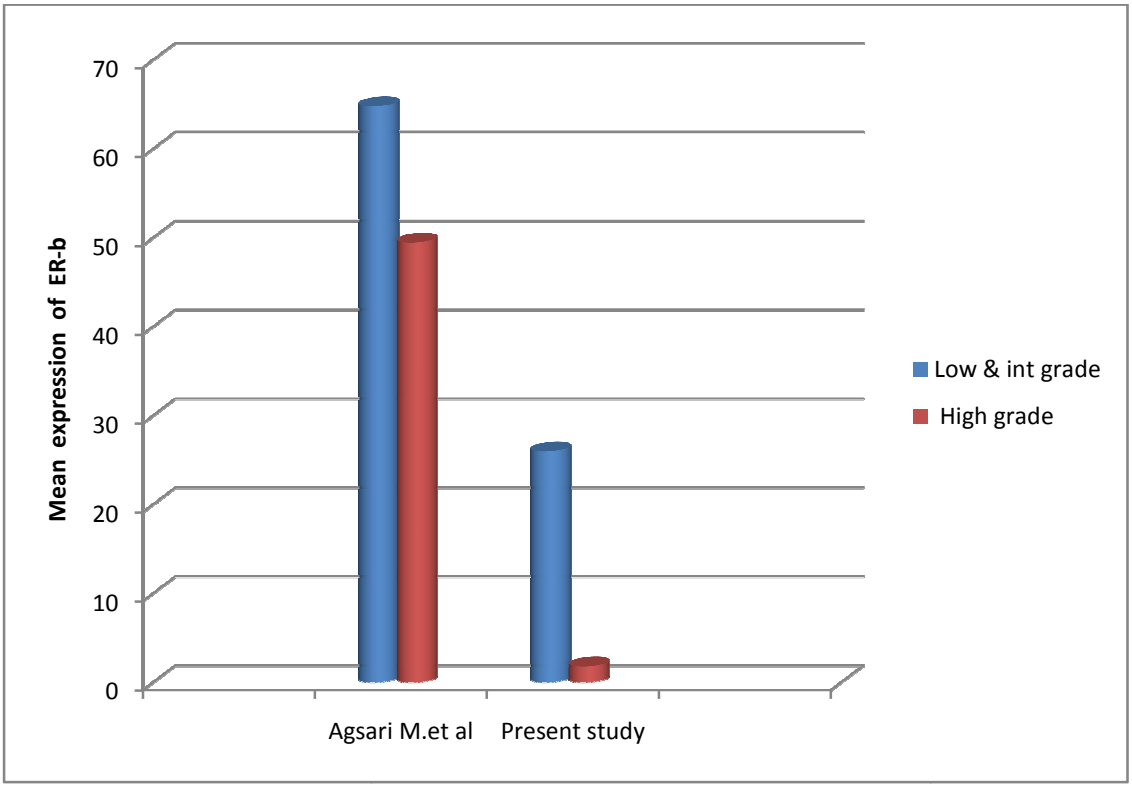
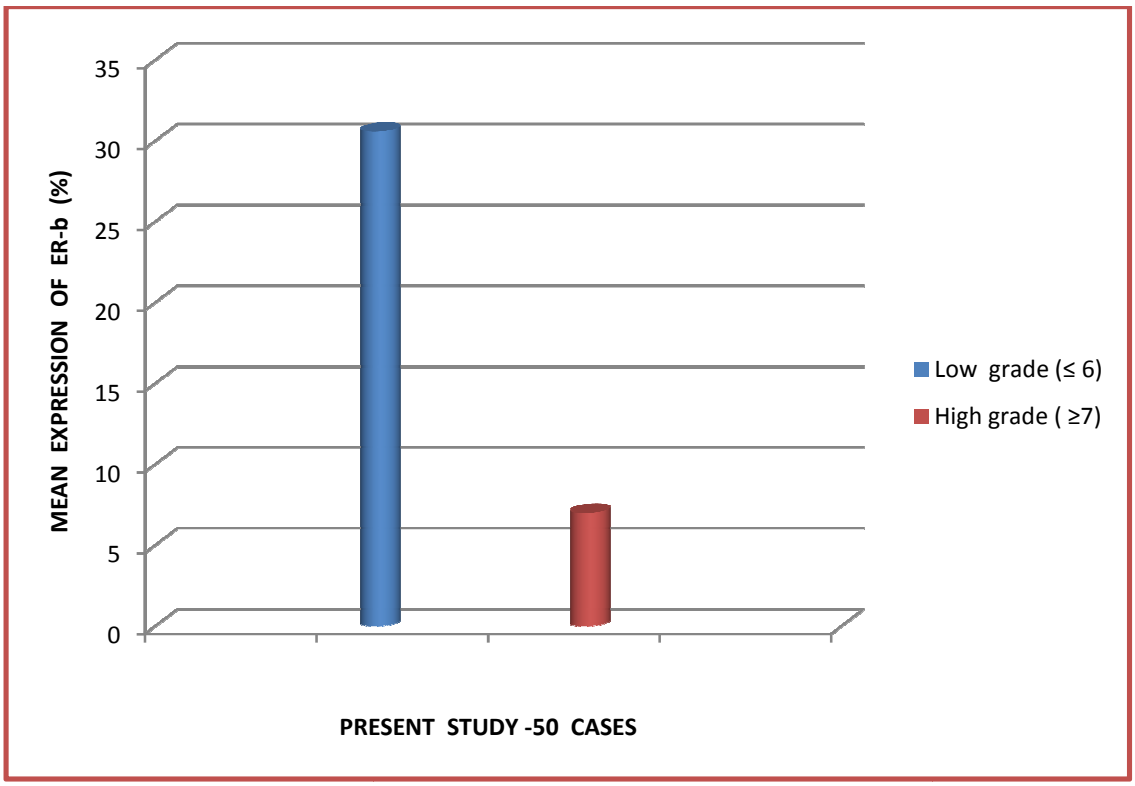


Fig 57 -COMPARISON OF MEAN EXPRESSION OF ER-b BETWEEN LOW AND HIGH GRADE TUMORS USING MODIFIED GLEASON'S SCORING SYSTEM



The number of low grade tumors (85.29%) showing ER-b expression is higher when compared to the number of high grade tumors (31.25) The mean expression of ER b in low grade tumors (34 cases) is 30.58% when compared to the mean expression in high grade tumors (16 cases) which have a mean ER-b expression of 7%

Thus, this study shows that ER- b expression is reduced in high grade tumors when compared to low grade tumors.

Figures 58- 61 show nuclear expression of ER-b by low grade carcinomas.

Figures 63- 66 show high grade prostate adenocarcinomas with lack of ER-b expression.

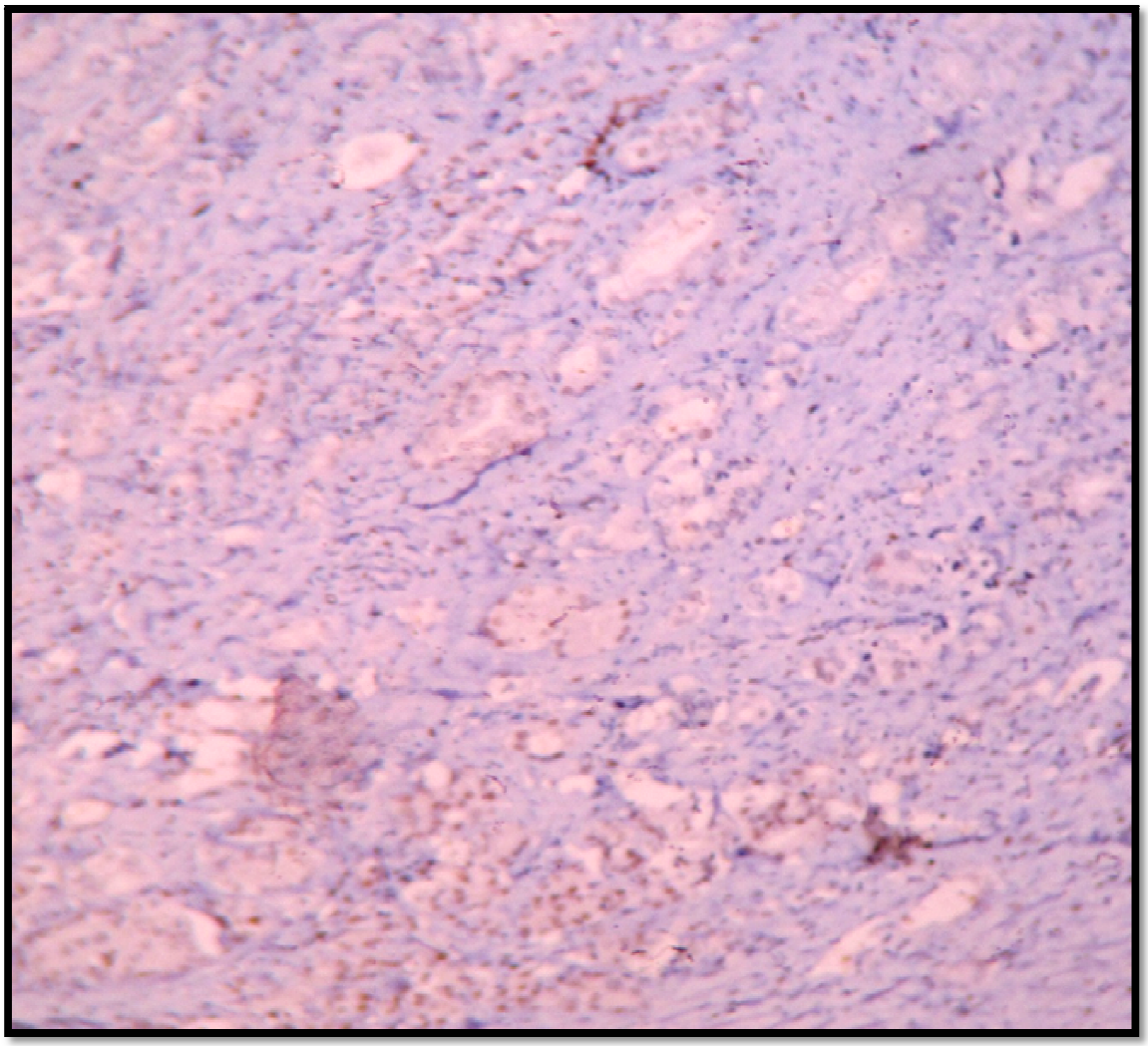


Fig .58 -Nuclear expression of ER-b in low grade tumor (3+2=5) -10x

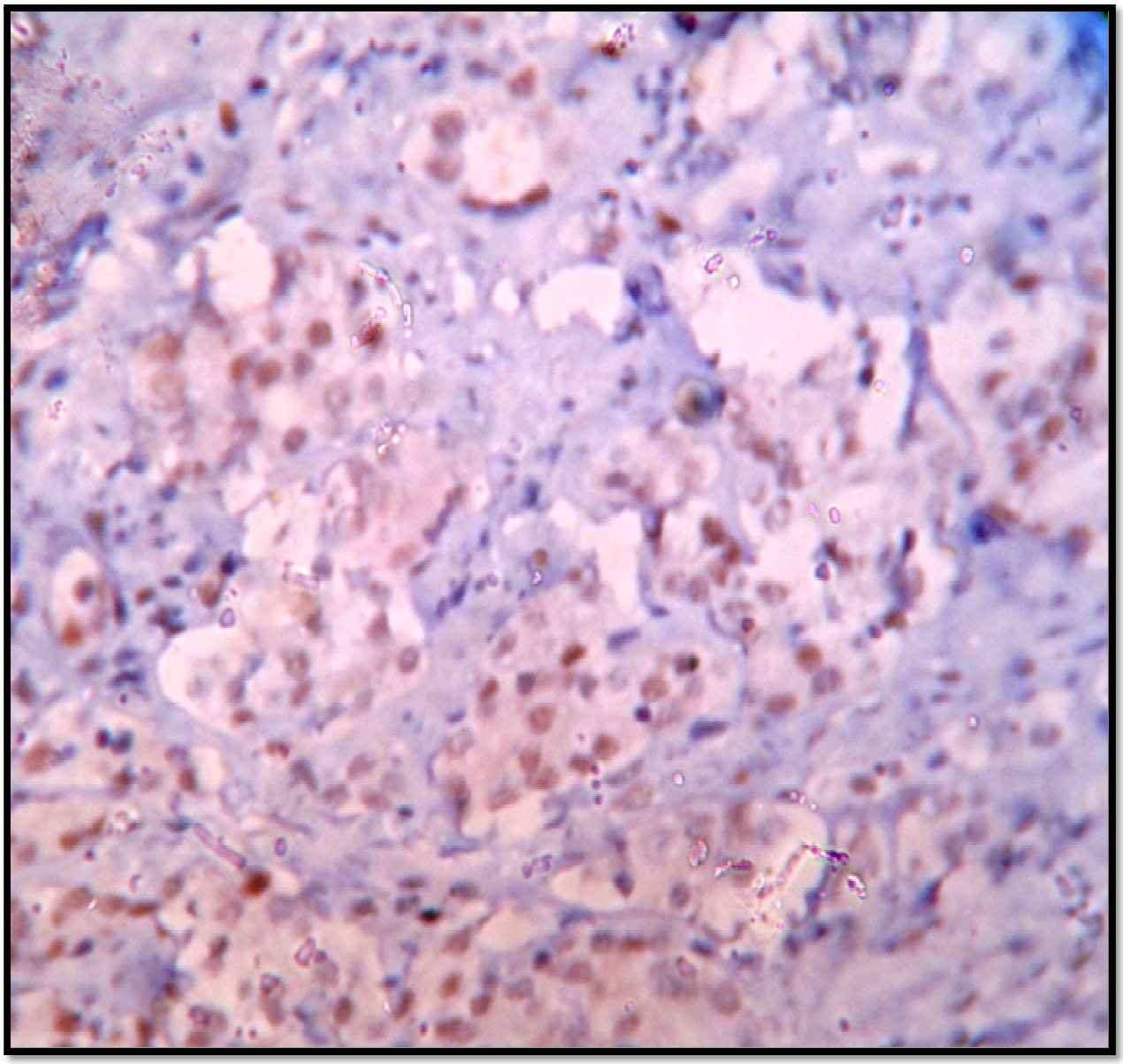


Fig.59- Nuclear expression of ER-b in low grade tumor (3+2=5) - 40x

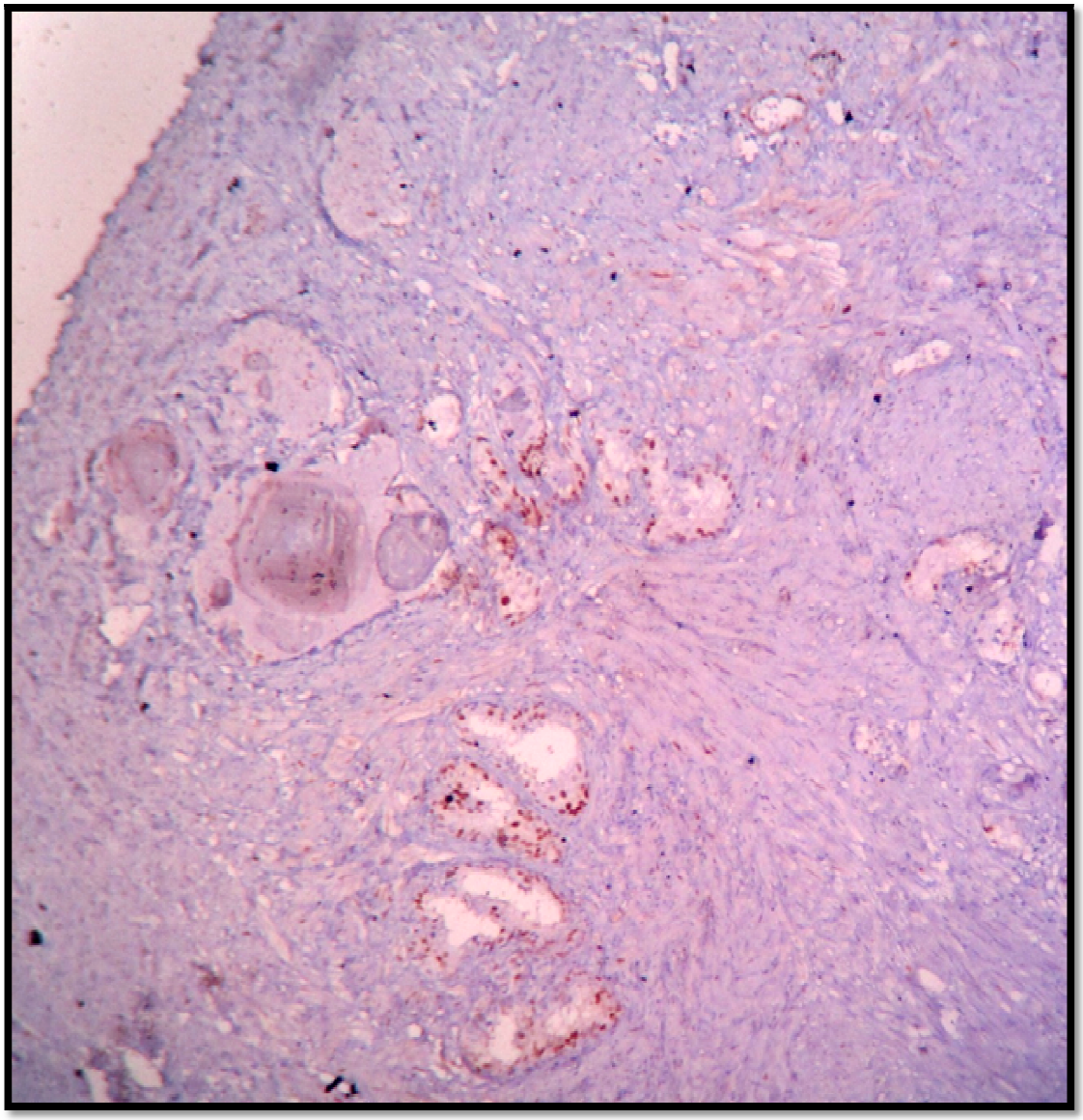


Fig.60 Nuclear expression of ER-b in low grade tumor (2+1=3)

10x

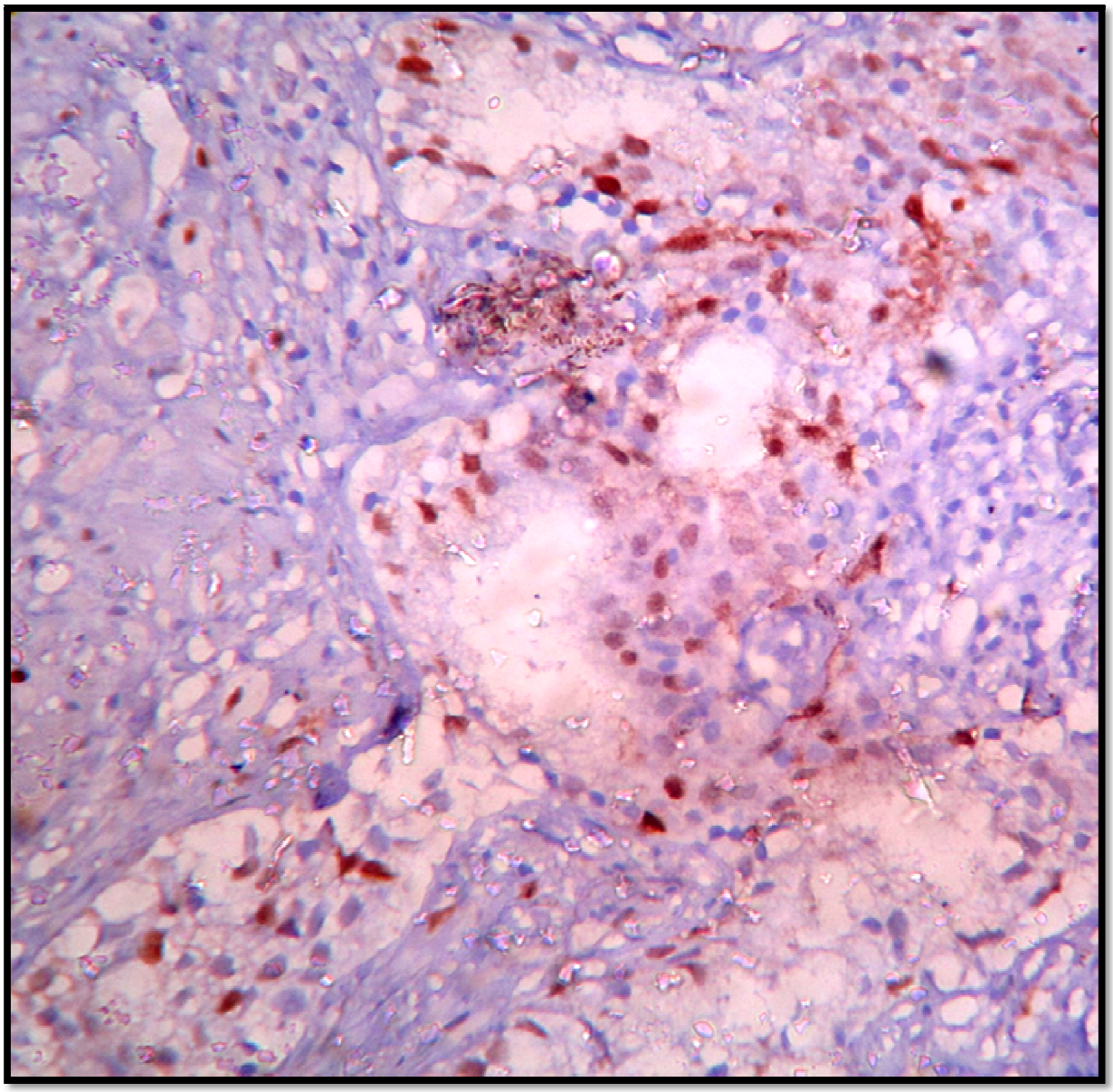


Fig.61 -Nuclear expression of ER-b in low grade tumor (2+1=3) -40x

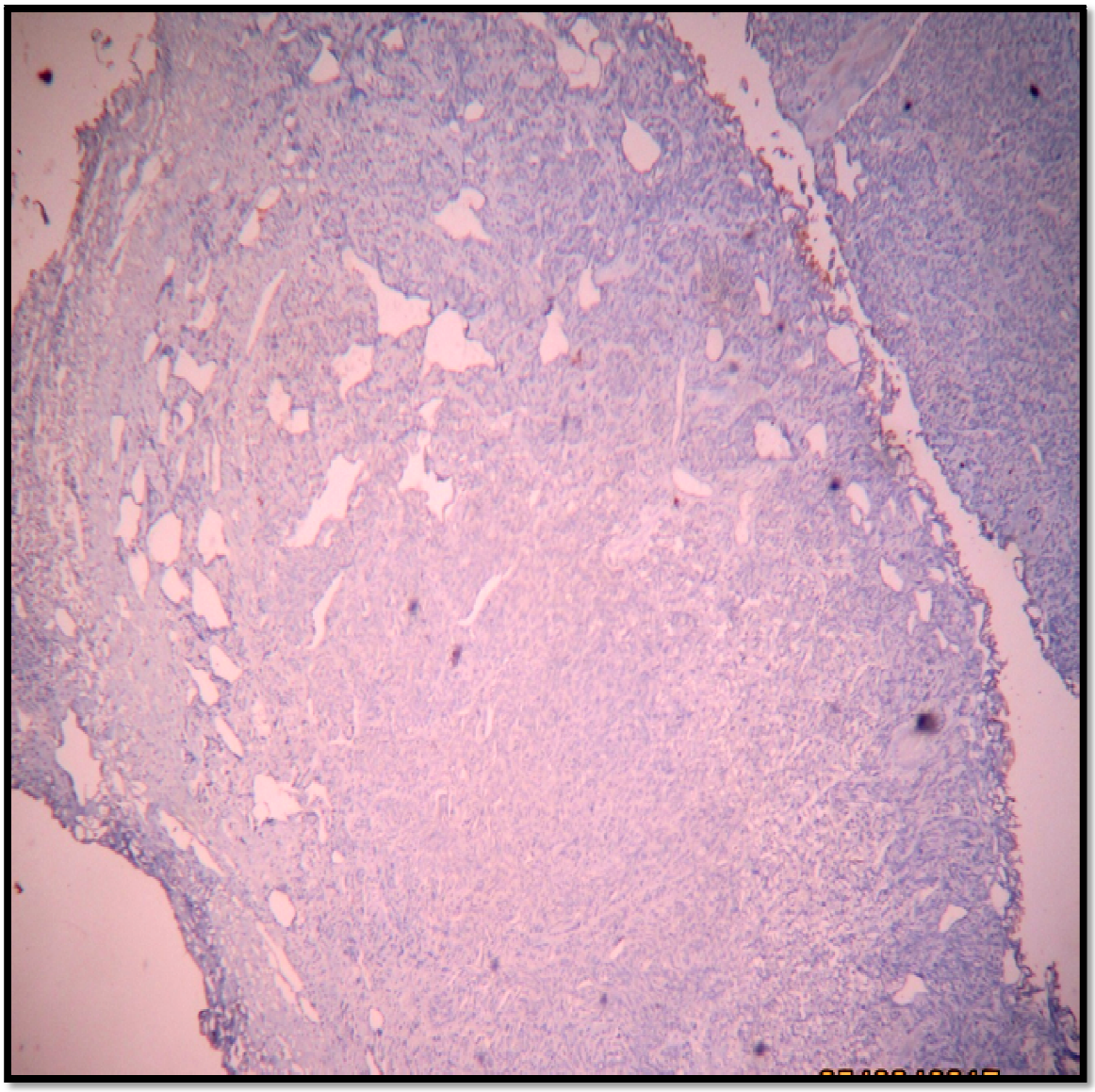


Fig.62 -Lack of nuclear expression of ER-b in a high grade prostate adenocarcinoma

(4+5= 9) - 10x

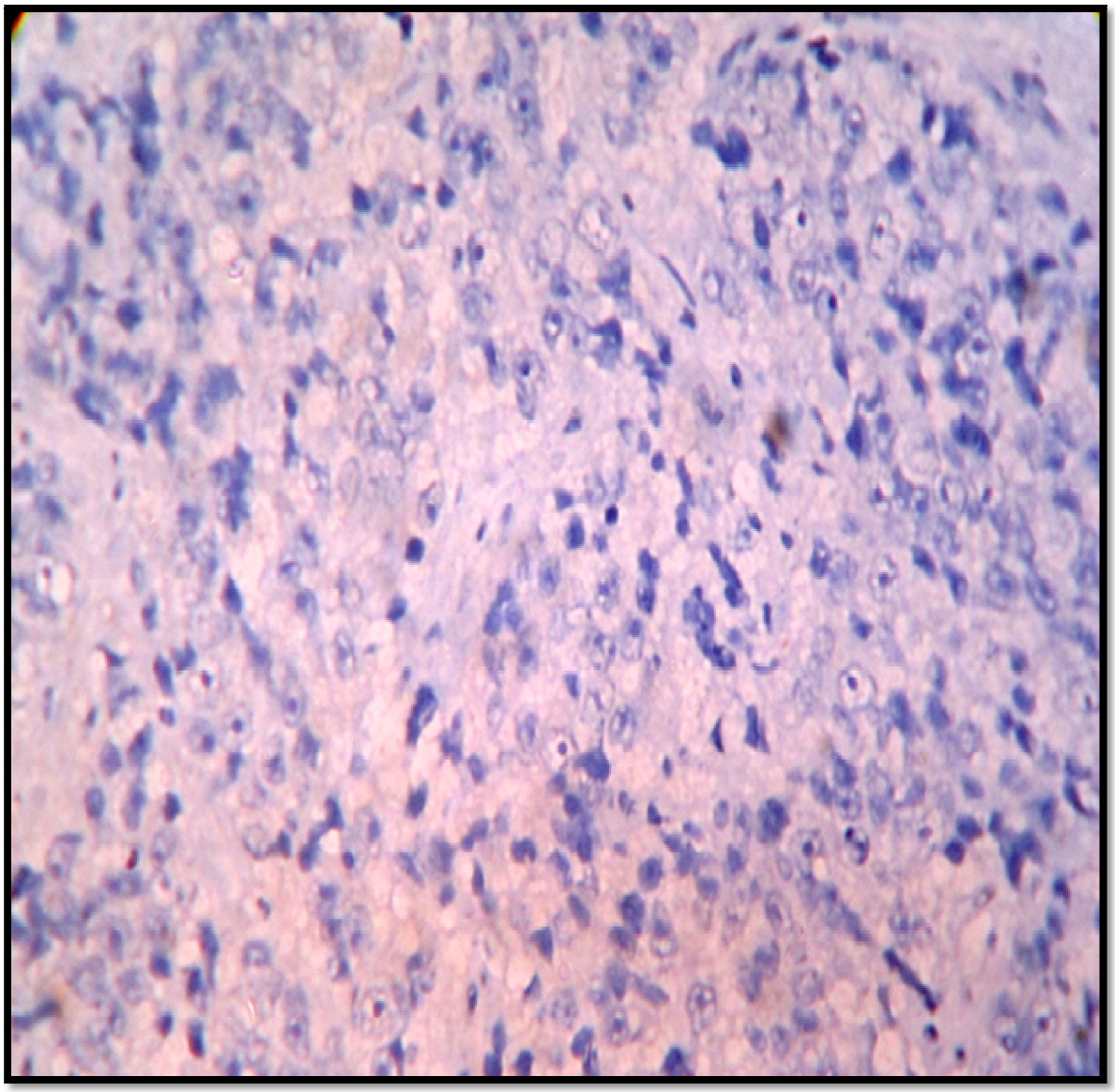


Fig.63- Lack of nuclear expression of ER-b in a high grade prostate adenocarcinoma

(4+5= 9) - 40x

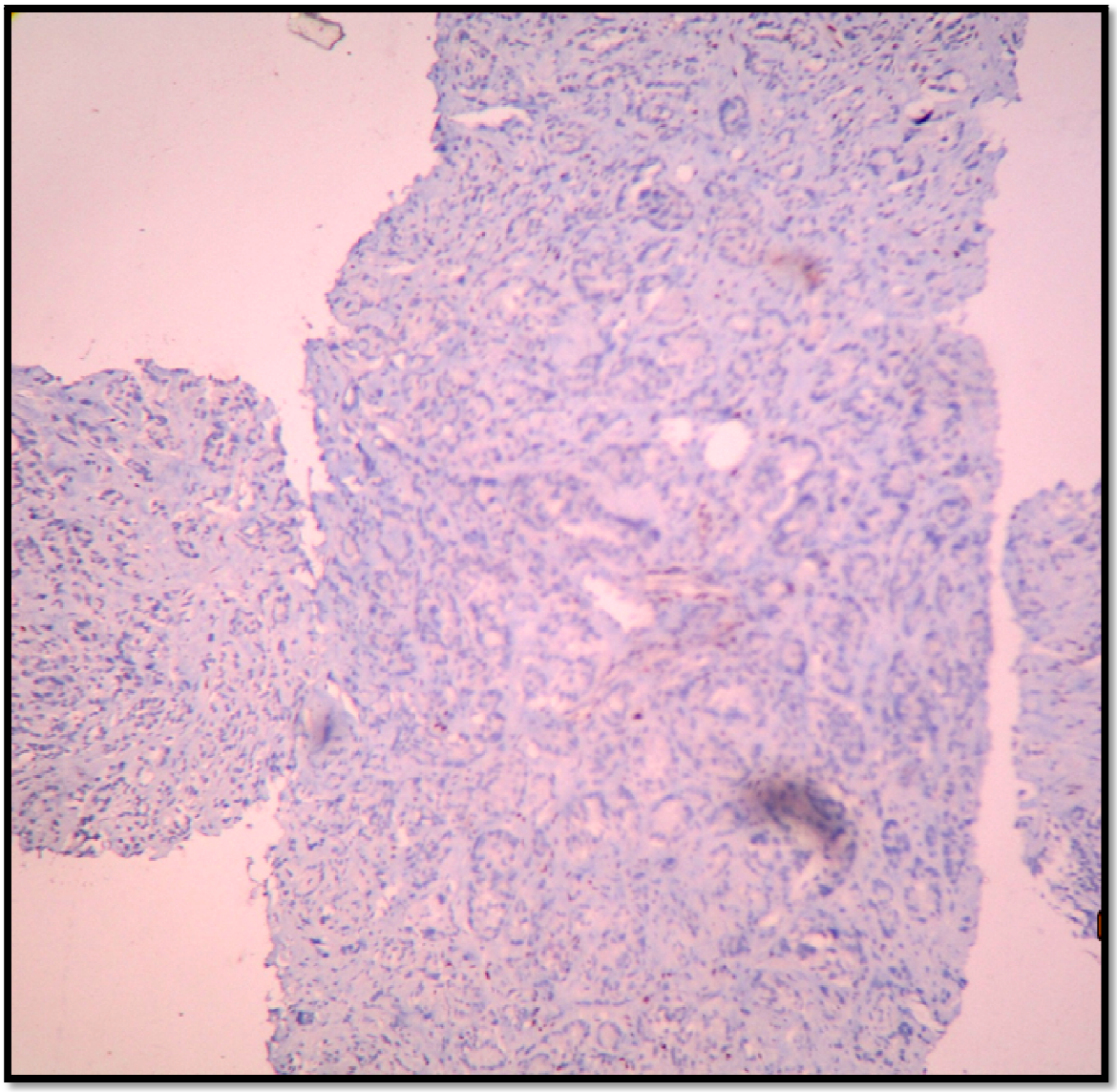


Fig.64 -Lack of nuclear expression of ER-b in a high grade prostate adenocarcinoma (5+4=9)- 10x

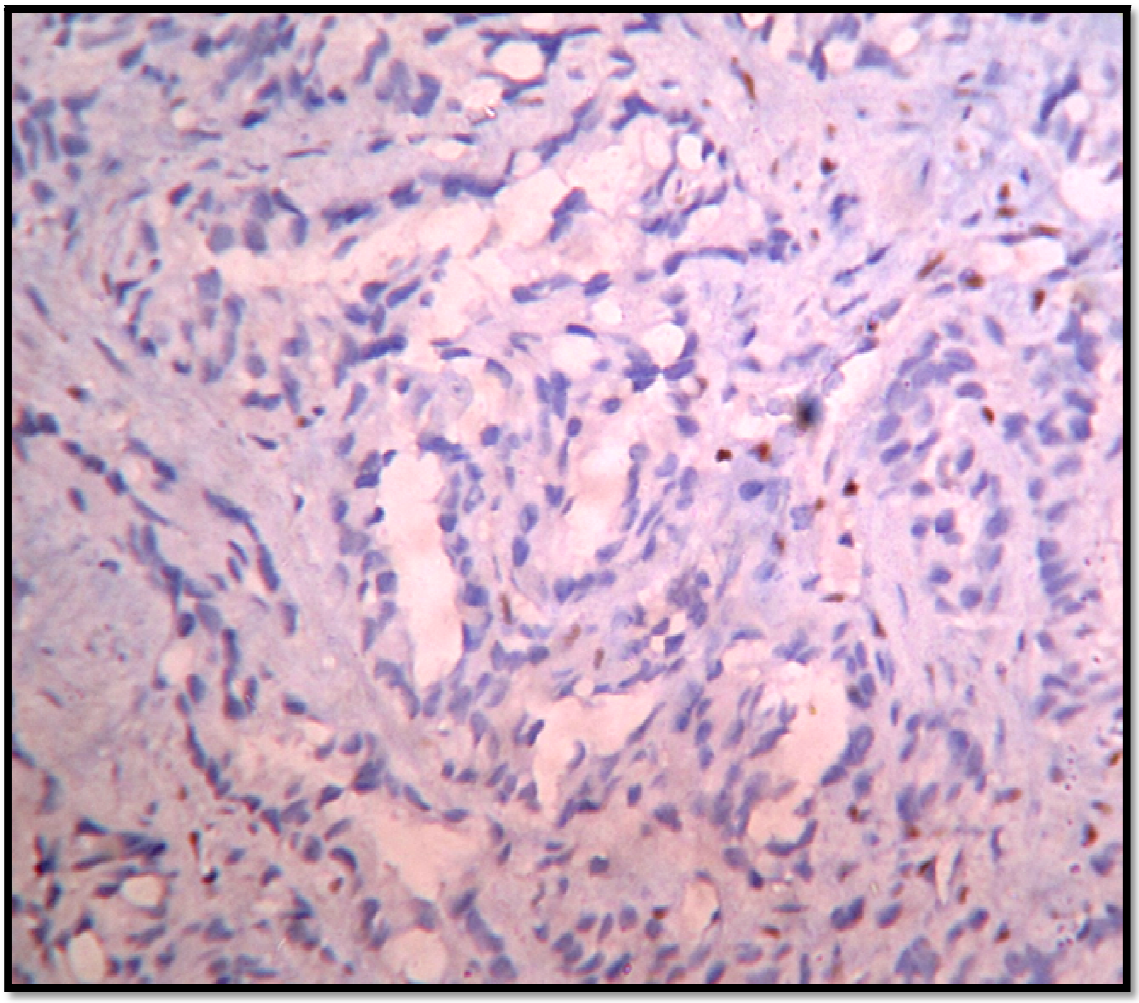


Fig.65- Lack of nuclear expression of ER-b in a high grade prostate adenocarcinoma (5+4=9)- 40x

ROLE OF ALCIAN BLUE STAIN IN PROSTATE ADENOCARCINOMAS

Alcian blue stain is used to demonstrate the acid mucin production by prostate adenocarcinomas.

Khanna A. et al conducted a study to assess the potentiality of pathological stains in human prostate cancer.6/9 (66.66%) primary prostate carcinomas were positive for Alcian blue stain at pH 2.5. Blue colored acid mucin was observed. 3 /9 (33.33%) cases that showed alcian blue positivity had a Gleason score ≤ 3 and the remaining 3 /9 (33.33%) cases had a score ≥ 4.Thus, it was stated that the secretory capacity of synthesizing acid mucin by the malignant cells with higher grades.[43]

Another study was conducted by Agarwal ND et al and the usefulness of mucin stains in the lesions of prostate was evaluated. Sections from 30 cases of prostate carcinoma were obtained. The sections were stained with Alcian blue at pH 2.5. All cases of low grade prostate adenocarcinomas showed positivity for acid mucin and none of the high grade carcinomas showed positivity for the same.

7/7 (100%) well differentiated and 7/7 (100%) intermediate grade tumors showed acid mucin production that was demonstrated with alcian blue at pH 2.5. None (0/7) of the moderate - poorly differentiated and (0/9) high grade tumors showed acid mucin production. Thus, the production of acid mucin by the tumor decreases with increase in the grade.[45]

Mathur SK et al also studied the significance of mucin stain in the lesions of prostate . Acid mucin was found in 68% of prostate carcinoma cases . The positivity for acid mucin was more in well differentiating tumour when compared to high grade malignancies.[44]

Also a study was conducted by Arora HL et al in 1979 and it was found that 60% of prostate carcinoma cases showed acid mucin secretion that was demonstrated by alcian blue stain at pH 2.5.[46]

Similar studies were conducted by Pinder et al and McMohan et al and acid mucin secretion was demonstrated by alcian blue stain at pH 2.5. 20/53 cases (38%) showed significant acid mucin production.[47]

In the present study , 21/ 50 cases (42%) showed acid mucin production. Out of the 34 low grade tumors , 20 cases showed acid mucin positivity (59%).Out of the 8 tumors with intermediate grade (3+4=7) , 1 case (12.5%) showed acid mucin production. None of the moderately to poorly differentiated tumors (4+3=7) and undifferentiated high grade tumors (score 8-10) showed acid mucin production.

Thus, this study shows that secretory capacity of the tumor decreases as the grade of tumor increases. This finding is similar to the study conducted by Agarwal ND et al.

Figure 66 shows a comparison between different grades of prostate adenocarcinomas showing acid mucin production.

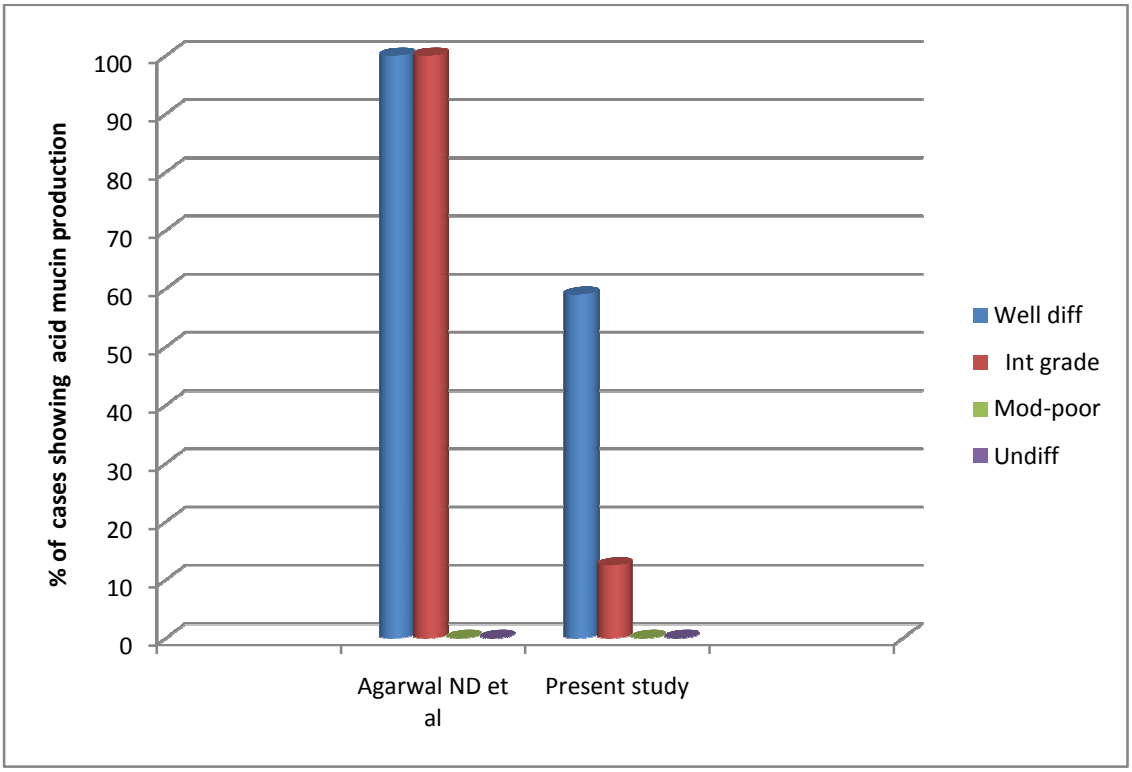
Figure 67 shows alcian blue positivity in a low grade tumor.

Figure 68 shows a high grade tumor showing no acid mucin secretion.

S.NO	AUTHORS	% OF PROSTATE CANCER CASES SHOWING ACID MUCIN POSITIVITY.
1.	Khanna A .et al	66.67
2.	Agarwal ND et al	46.66
3.	Arora HL et al	60
4.	Pinder et al	38
5.	McMahon et al	50
6.	Mathur SK et al	68
7.	Present study	42

GRADE OF TUMOR	NO OF CASES	NO OF CASES SHOWING ACID MUCIN	P -value
Low grade (2-6)	34	20 (59%)	p < 0.001
Intermediate grade (3+4)	8	1 (12.5%)	
Moderate to poor differentiated (4+3)	2	0	
High grade tumors (8,9,10)	6	0	

Fig .66 -COMPARISON OF ALCIAN BLUE POSITIVITY BETWEEN DIFFERENT GRADES OF PROSTATE ADNOCARCINOMA



Thus , this study shows that acid mucin production gets reduced as the Gleason's score of the tumor increases.

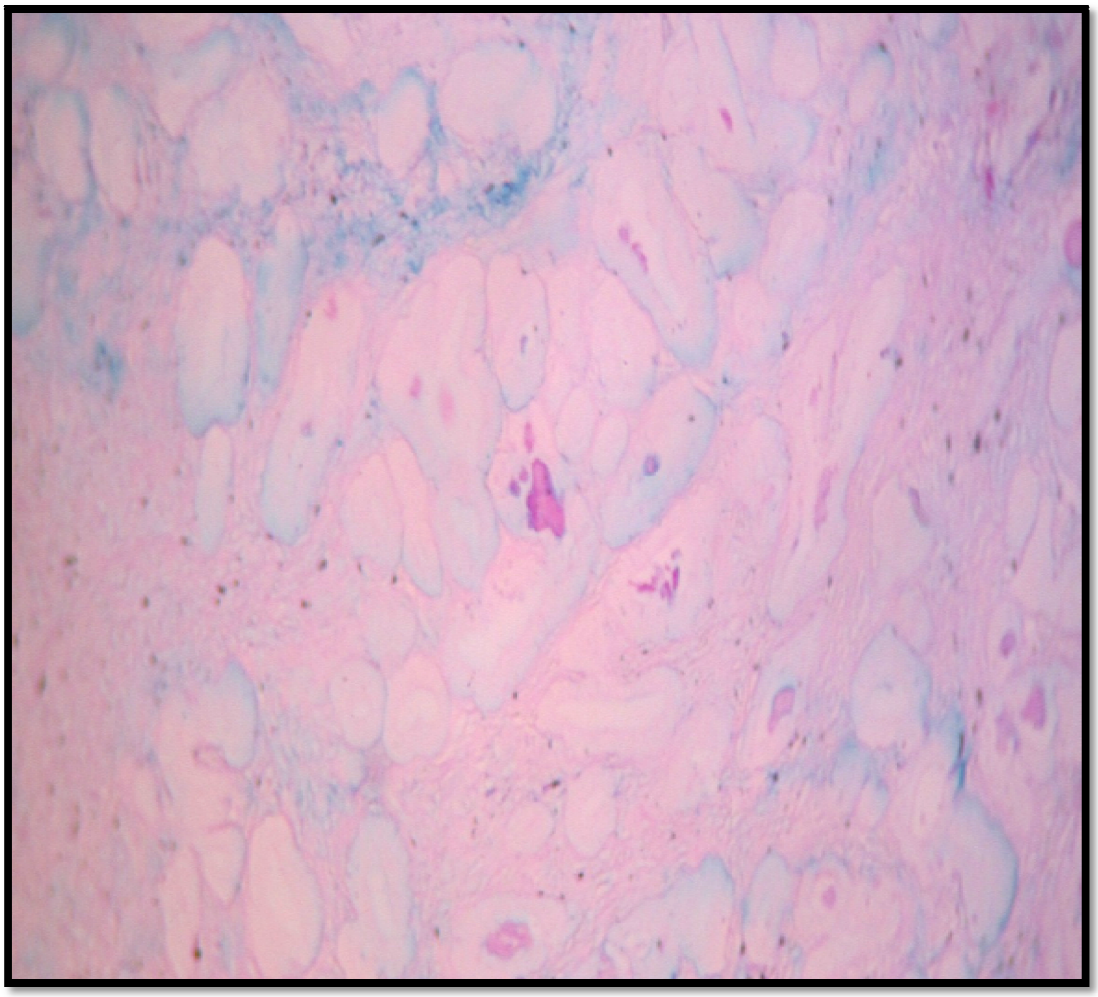


Fig 67. Alcian blue -PAS stain demonstrating acid mucin production in low grade prostate adenocarcinoma (3+2=5).

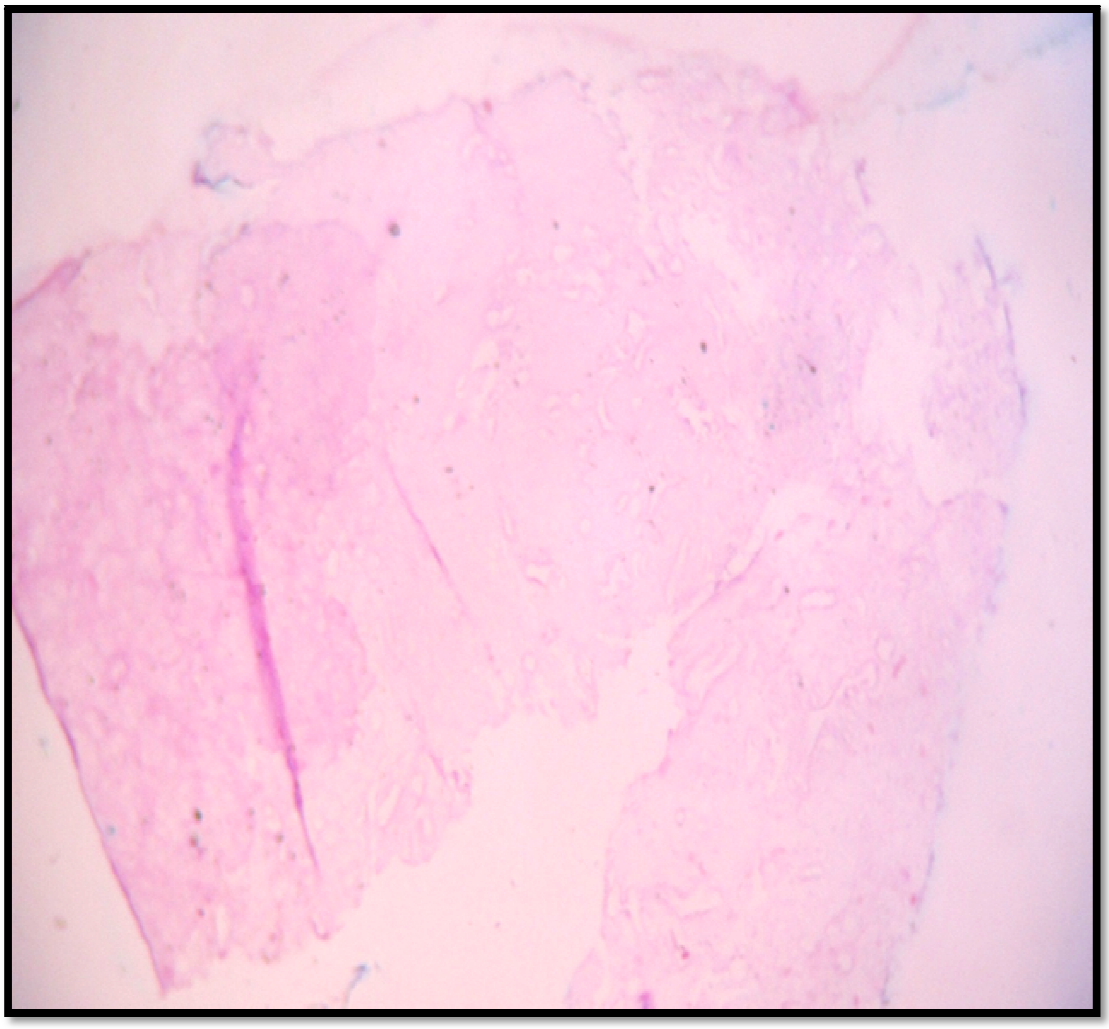


Fig.68 - Loss of acid mucin secretion in a high grade prostate adenocarcinoma (Grade 4+5=9)

CONCLUSION

CONCLUSION

1. Out of the 50 cases , 60% of the cases were between the age group of 70 - 80 years of age.

2. Peripheral zone was involved by the tumor in all the 50 cases included in the study . Transition zone was not involved in any of the cases.

3. There was a steep rise in the serum PSA level with increase in the Gleason's score. Mean serum PSA level in cases with low Gleason's score was 27.9 ng/mL .This was lower than serum PSA level of individuals with high Gleason score in whom it was 139.25 ng/mL.

4. A strong correlation was found between cyclin D1 expression and Gleason's score. All cases with high grade tumors (100%) showed cyclin D1 positivity . The mean expression of cyclin D1 was higher (35%) in high grade tumors than low grade tumors (16%).

5. A significant correlation between the grade of the tumor and Ki-67 positivity was found. 94 % of the high grade tumors showed Ki-67 expression as compared to low grade tumors where the expression of Ki-67 was found only in 29 % of the cases.

6. ER-b expression was found to be reduced in high grade tumors (7%) when compared to the low grade tumors which had a mean ER-b expression of 30.58%

7. 59% of the well differentiated tumors showed acid mucin production. None of the poorly differentiated tumors secreted acid mucin. Thus, this shows that the secretory capacity of the tumor reduces with increasing Gleason's score.

ANNEXURE

ANNEXURE-I

Histopathological analysis of prostate adenocarcinoma and role of immunohistochemistry in distinction between low grade and high grade carcinomas.

CASE DETAILS PROFORMA

- Name of the patient:
- Age:
- OP/IP No:
- Chief complaints :
- Digital rectal examination findings:
- Radiological findings:
- Serum PSA levels:
- Histopathology No:

HISTOPATHOLOGICAL EXAMINATION

Major criteria of prostate adenocarcinoma

- Architecture of the glands: presence or absence of infiltrative small glands.
- Presence or absence of basal cell layer.
- Presence or absence of nuclear atypia.
- Nucleolar enlargement.

Minor criteria

- Intraluminal wispy mucin.
- Pink amorphous secretions.
- Mitotic figures.
- Intraluminal crystalloids.
- Adjacent high grade PIN.
- Amphophilic cytoplasm.

ANNEXURE -II

IMMUNOHISOCHEMISTRY PROTOCOL

1. Place the slides in microwave oven at 80 degree celsius for 3 mins . This step is done thrice.

2. Deparaffinize the sections twice in xylene for 3 mins.

3. Dexylenize the sections with 100% isopropyl alcohol.

4.Bring sections to running tap water for 5 mins.

5.Antigen retrieval solution is prepared using sodium citrate.

6. Wash buffer is prepared .

7. Sections are heated in a pressure cooker containing antigen retrieval solution.

8. Sections are allowed to cooled.

9. Bring sections to running tap water.

10.Wash with distilled water for 2 mins.

11. Put peroxidase block- 5 mins.

12.Place the sections in wash buffer - 2 mins.

12. Bring the sections to running tap water -2 to 3 mins

13. Wash with distilled water - 2 mins, 2 washes

14. Add primary antibody- one and half hour

15. Place the sections in wash buffer - 2 mins, 2 washes

16. Put polymer HRP - 30 mins.

17.Wash with wash buffer (PBS) - 2 mins, 3 washes.

18. Add DAB chromogen

19.Wash with PBS - two changes.

20.Stain with Hematoxylin - 2 mins

21. Dip in acid alcohol.

22. Blueing.

23. Dry , mount.

24. Observe under light microscope.

ANNEXURE - III

COMBINED ALCIAN BLUE- PAS TECHNIQUE

✓ ALCIAN BLUE SOLUTION:

- Alcian blue 8GX - 1g
- 3% acetic acid solution - 100 ml

✓ PERIODIC ACID SOLUTION:

- Periodic acid -1 g
- Distilled water- 100 ml

✓ PREPARATION OF SCHIFF REAGENT:

Dissolve 1g of basic fuchsin and 1.9 g of sodium metabisulfite in 100 ml of 0.15 M hydrochloric acid (HCl). Shake the solution at intervals or on a mechanical shaker for 2 hours. The solution should be clear and yellow to light brown in colour. Add 500 mg of activated charcoal and shake for 1-2 mins. Filter the solution .The filtered solution should be clear and colourless. Store in brown/ amber colored bottle at 4 degree celsius.

COMBINED ALCIAN BLUE -PAS TECHNIQUE

1. Dewax the sections in xylene and rehydrate through graded ethanols to distilled water.

2.Stain in alcian blue solution for 30 mins.

3. Rinse in running tap water for 5 mins and then briefly in distilled water.

4. Oxidize with periodic acid for 5 mins.

5. Rinse in running tap water water 5 mins.

6. Cover the sections with Schiff reagent for 15 mins.

7. Rinse in running tap water for 10 mins.

8. Stain lightly with hematoxylin.

9. Rinse in running tap water for 5-10 mins and blue in an appropriate blueing solution.

10. Rinse in tap water for 5 mins.

11. Dehydrate in graded ethanols, clear with xylene , and mount.

RESULTS:

ACID MUCIN - BLUE

NEUTRAL MUCIN - MAGENTA.

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MASTER CHART

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	NO.	AGE	SYMPTOM	ZONE INVOLVED	S. PSA (ng/mL)	GLEASON SCORE	CYCLIND1	Ki67	ER	Alcian blue - PAS
1	7/15	75	Dribbling of urine	PERIPHERAL	20	(1+1) 2	<5%	<2%	60%	Positive
2	69/15	60	Dribbling of urine	PERIPHERAL	25	(2+1) 3	<5%	<2%	50%	Positive
3	137/15	72	Retention	PERIPHERAL	100	(3+3) 6	10%	2-25%	20%	Negative
4	177/15	65	Frequency	PERIPHERAL	30	(3+2) 5	<5%	>2%	30%	Positive
5	180/15	70	Hesitancy	PERIPHERAL	26.5	(3+2) 5	<5%	<2%	40%	Positive
6	236/15	65	Retention	PERIPHERAL	160	(3+4) 7	20%	2-25%	20%	Negative
7	675/15	70	Hesitancy	PERIPHERAL	30	(2+2) 4	<5%	<2%	40%	Positive
8	1359/15	65	Frequency	PERIPHERAL	24	(2+2) 4	<5%	<2%	50%	Positive
9	1564/15	70	Dribbling	PERIPHERAL	36	(3+2) 5	<5%	<2%	<10%	Positive
10	1663/15	65	Back pain	PERIPHERAL	85	(3+3) 6	<5%	<2%	20%	Positive
11	1666/15	75	Urgency	PERIPHERAL	240	(5+4) 9	60%	26-50%	<10%	Negative

12	**1716/15**	**70**	**Nocturia**	**PERIPHERAL**	**100**	**(3+3) 6**	**10%**	**2-25%**	**20%**	**Negative**
13	**1900/15**	**75**	**Frequency**	**PERIPHERAL**	**30**	**(3+2) 5**	**<5%**	**2-25%**	**30%**	**Positive**
14	**2109/15**	**70**	**Retention**	**PERIPHERAL**	**15**	**(1+1) 2**	**<5%**	**<2%**	**80%**	**Positive**
15	**2170/15**	**50**	**Nocturia**	**PERIPHERAL**	**20**	**(2+2) 4**	**<5%**	**<2%**	**40%**	**Positive**
16	**2493/15**	**73**	**Hematuria**	**PERIPHERAL**	**350**	**(4+5) 9**	**50%**	**51-75%**	**<10%**	**Negative**
17	**3006/15**	**75**	**Frequency**	**PERIPHERAL**	**15.5**	**(2+2) 4**	**<5%**	**<2%**	**40%**	**Positive**
18	**3101/15**	**60**	**Urgency**	**PERIPHERAL**	**20**	**(2+1) 3**	**<5%**	**<2%**	**50%**	**Positive**
19	**3323/15**	**60**	**Back pain**	**PERIPHERAL**	**100**	**(3+4) 7**	**20%**	**2-25%**	**<10%**	**Negative**
20	**3354/15**	**60**	**Retention**	**PERIPHERAL**	**110**	**(4+3) 7**	**30%**	**26-50%**	**<10%**	**Negative**
21	**3411/15**	**78**	**Frequency**	**PERIPHERAL**	**30**	**(2+1) 3**	**<5%**	**<2%**	**50%**	**Positive**
22	**3499/15**	**75**	**Urgency**	**PERIPHERAL**	**210**	**(4+5) 9**	**50%**	**26-50%**	**<10%**	**Negative**
23	**3839/15**	**70**	**Urgency**	**PERIPHERAL**	**30**	**(2+2) 4**	**<5%**	**<2%**	**<10%**	**Positive**
24	**3909/15**	**65**	**Frequency**	**PERIPHERAL**	**35.7**	**(3+2) 5**	**<5%**	**<2%**	**30%**	**Negative**
25	**3915/15**	**70**	**Hesitancy**	**PERIPHERAL**	**170**	**(4+5) 9**	**50%**	**26-50%**	**<10%**	**Negative**
26	**3939/15**	**75**	**Urgency**	**PERIPHERAL**	**50**	**(3+2) 5**	**<5%**	**<2%**	**<10%**	**Positive**
27	**4273/15**	**80**	**Retention**	**PERIPHERAL**	**40**	**(3+2) 5**	**<5%**	**2-25%**	**30%**	**Negative**
28	**165/16**	**72**	**Frequency**	**PERIPHERAL**	**100**	**(3+4)7**	**20%**	**2-25%**	**20%**	**Negative**

29	**280/16**	**75**	**Retention**	**PERIPHERAL**	**30**	**(3+2) 5**	**<5%**	**2-25%**	**40%**	**Positive**
30	**366/16**	**80**	**Urgency**	**PERIPHERAL**	**280**	**(4+5) 9**	**50%**	**26-50%**	**<10%**	**Negative**
31	**876/16**	**75**	**Hematuria**	**PERIPHERAL**	**80**	**(3+3) 6**	**<5%**	**2-25%**	**<10%**	**Negative**
32	**979/16**	**80**	**Back pain**	**PERIPHERAL**	**95**	**(3+3) 6**	**10%**	**<2%**	**20%**	**Positive**
33	**1408/16**	**74**	**Urgency**	**PERIPHERAL**	**110**	**(3+4) 7**	**20%**	**2-25%**	**20%**	**Negative**
34	**1430/16**	**75**	**Frequency**	**PERIPHERAL**	**30**	**(3+2) 5**	**<5%**	**2-25%**	**40%**	**Negative**
35	**1821/16**	**75**	**Nocturia**	**PERIPHERAL**	**100**	**(3+4) 7**	**20%**	**2-25%**	**20%**	**Negative**
36	**3414/16**	**80**	**Retention**	**PERIPHERAL**	**40**	**(3+2) 5**	**<5%**	**<2%**	**40%**	**Negative**
37	**3415/16**	**70**	**Frequency**	**PERIPHERAL**	**90**	**(3+3) 6**	**<5%**	**2-25%**	**<10 %**	**Negative**
38	**4002/16**	**75**	**Retention**	**PERIPHERAL**	**130**	**(3+4) 7**	**30%**	**<2%**	**<10%**	**Negative**
39	**4477/16**	**74**	**Urgency**	**PERIPHERAL**	**90**	**(3+3) 6**	**<5%**	**<2%**	**30%**	**Negative**
40	**4617/16**	**70**	**Retention**	**PERIPHERAL**	**80**	**(3+3) 6**	**15%**	**2-25%**	**30%**	**Negative**
41	**4632/16**	**74**	**Back pain**	**PERIPHERAL**	**110**	**(3+3) 6**	**20%**	**<2%**	**30%**	**Negative**
42	**4666/16**	**75**	**Hematuria**	**PERIPHERAL**	**30**	**(3+2) 5**	**10%**	**<2%**	**30%**	**Negative**
43	**4789/16**	**72**	**Retention**	**PERIPHERAL**	**30**	**(3+2) 5**	**<5%**	**<2%**	**30%**	**Negative**
44	**184/17**	**75**	**Urgency**	**PERIPHERAL**	**160**	**(3+4) 7**	**30%**	**2-25%**	**30%**	**Negative**
45	**337/17**	**80**	**Nocturia**	**PERIPHERAL**	**140**	**(3+4) 7**	**30%**	**2-25%**	**<10%**	**Positive**

46	**451/17**	**70**	**Frequency**	**PERIPHERAL**	**40**	**(3+2) 5**	**20%**	**<2%**	**30%**	**Negative**
47	**455/17**	**75**	**Retention**	**PERIPHERAL**	**110**	**(4+3)7**	**40%**	**2-25%**	**<10%**	**Negative**
48	**517/17**	**78**	**Retention**	**PERIPHERAL**	**90**	**(3+3) 6**	**30%**	**2-25%**	**20%**	**Positive**
49	**1240/17**	**75**	**Hematuria**	**PERIPHERAL**	**270**	**(5+4) 9**	**40%**	**51-75%**	**<10%**	**Negative**
50	**2809/17**	**73**	**Retention**	**PERIPHERAL**	**100**	**(3+3) 6**	**<5%**	**<2%**	**20%**	**Positive**